Conformational and Functional Differences between Recombinant Human Lens αA- and αB-Crystallin*

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Human and other mammalian lens proteins are composed of three major crystallins: α-, β-, and γ-crystallin. α-Crystallin plays a prominent role in the supramolecular assembly required to maintain lens transparency. With age, the crystallins, especially α-cristallin, undergo posttranslational modifications that may disrupt the supramolecular assembly, and the lens becomes susceptible to other stresses resulting in cataract formation. Because these modifications occur even at a relatively young age, it is difficult to obtain pure, unmodified crystallins for in vitro experiments. α-Cristallin is composed of two subunits, αA and αB. Before the application of recombinant DNA technology, these two α-cristallin subunits were separated from calf lens in the denatured state and reconstituted by the removal of the denaturant, but they were not refolded properly. In the present studies, we applied the recombinant DNA technology to prepare native, unmodified αA- and αB-cristallins for conformational and functional studies. The expressed proteins from Escherichia coli are in the native state and can be studied directly. First, αA and αB cDNAs were isolated from a human lens epithelial cell cDNA library. The cDNAs were cloned into a pAED4 expression vector and then expressed in E. coli strain BL21(DE3). Pure recombinant αA- and αB-cristallins were obtained after purification by gel filtration and DEAE liquid chromatography. They were subjected to conformational studies involving various spectroscopic measurements and an assessment of chaperone-like activity. αA- and αB-cristallins have not only different secondary structure, but also tertiary structure. 1-Anilino-8-naphthalene sulfonate fluorescence indicates that αB-cristallin is more hydrophobic than αA-cristallin. The chaperone-like activity, as measured by the ability to protect insulin aggregation, is about 4 times greater for αB- than for αA-cristallin. The resulting data provide a base line for further studies of human lens α-cristallin.

α-Crystallin is the major lens structural protein responsible for the supramolecular assembly necessary to maintain lens transparency. It is isolated as a large water-soluble aggregate with a mass of 800 kDa and is composed of two homologous subunits, αA and αB, each with a molecular mass of 20 kDa. With aging and cataract formation, α-crystallins gradually be- come a high molecular weight aggregate and eventually an insoluble protein (1). This is manifested by the observation that the major crystallin component in the insoluble protein fraction of aged and cataractous lenses is α-cristallin (2). The aggregation and insolubilization of α-cristallin are believed to arise from posttranslational modifications, which accumulate significantly because of the low turnover rate of lens crystallins. Two-dimensional gel electrophoresis indicated that human lens crystallins were modified extensively even at a relatively young age (3). The significance of the modification is that crystallins become partially unfolded, and this partially unfolding exposes hydrophobic surfaces and promotes hydrophobic interaction. In aged and cataractous lenses, aggregation and insolubilization take place because the concentration of partially unfolded protein is high.

Currently, most human lens specimens available for in vitro studies are from old human subjects or cataract surgery, and the crystallins, especially α-cristallin isolated from these specimens, are extensively modified. Therefore, it is difficult to obtain pure and unmodified human lens α-cristallin for in vitro investigations. An alternative is to use an animal model, but this option raises concern about whether the results are species-specific or can be extrapolated to humans. For these reasons, many investigators used recombinant DNA technology to clone lens α-cristallin (4–11). The technique was employed to prepare not only unmodified α-cristallin but also site-specific mutants to study the effect of site-specific mutation on protein conformation and function. These studies, however, dealt only with either αA-cristallin (4–8, 10, 11) or αB-cristallin (9). Moreover, no comparative study on conformation and function between recombinant αA- and αB-cristallin has been reported. In the present study, we prepared both recombinant human αA- and αB-cristallins and characterized their conformations by molecular spectroscopy. The results provide a base line for further studies of modification-related changes in protein interaction and conformation. In addition, αA- and αB-cristallins were recently detected in other tissues, such as heart, kidney, spleen, and retina (12–15). These findings raise question about the role or function of αA- and αB-cristallins in these tissues. Similar studies of the corresponding recombinant αA- and αB-cristallins from tissues other than the lens may yield information on their conformation and aggregation state. This information may provide a clue to their biological functions.

MATERIALS AND METHODS

Isolation and Cloning of αA- and αB-Cristallin cDNA—A complementary DNA library of HLE cells prepared with a ZAP-cDNA Synthesis Kit (Stratagene, La Jolla, CA) was generously provided by Dr. Toshimichi Shinhara. The αA gene (689 bp) and the αB gene (657 bp)

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1 The abbreviations used are: bp, base pair(s); kb, kilobase pair(s); ANS, 1-anilino-8-naphthalenesulfonate; FPLC, fast performance liquid chromatography; IEF, isoelectric focusing; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction.
were isolated by PCR using two specific primers for each. The forward primer, d(AAGCTTGATATGCAGCTACCCACAAGCACAGC) for aα and d(ctacgcatatggCATCGGACATGGCCATCACC) for bβ, corresponds to a cDNA sequence containing an ATG translation start codon and incorporating a two base change (aAACAGTCATATGGCATATGGCCATCACC) for aα and a three-base change (aACACCATTGGCATATGGCCATCACC) for bβ to create a NdeI and HinIII restriction site. The reverse primer for aα, d(GACCCCTAAGCTTCTCGGCTGCTTCCAAACAC), corresponds to the cDNA sequence 125 nucleotides downstream of the AAG stop codon and incorporates a HinIII site. The reverse primer for bβ, d(TATAGCTTGATATGCTGGGCTGCTTAGGCCTATCACC) corresponds to the cDNA sequence 89 nucleotides downstream of the TAG stop codon and incorporates a NdeI restriction site. DNA ligation by DNA ligase.

30 cycles of 95°C (45 s), 55°C (1 min), and 72°C (2 min) each. The PCR product was purified by 2% agarose gel electrophoresis and was double-digested with NdeI and HinIII. The PCR products were then ligated into the two expression vectors pAED4, a 3322-bp ampicillin-resistant plasmid containing the restriction sites NdeI and HinIII at the polycloning sites (16). The primers were custom-synthesized by Life Technologies, Inc.

For PCR, we used a protocol for pBl DNA polymerase supplied by the manufacturer (Stratagene). The PCR mixture (10 ng of human lens epithelial cDNA, 200 pmol of each amplification primer, 10 μl of 10 × pBl DNA polymerase buffer, 20 nmol each of four dNTPs, and 2.5 units of pBl DNA polymerase in a final volume of 100 μl) was first heated to 95°C for 1 min to denature the template DNA; it was then subjected to 30 cycles of 95°C (45 s), 55°C (1 min), and 72°C (2 min) each. The PCR product was purified by 2% agarose gel electrophoresis and was double-digested with NdeI and HinIII. The PCR product was then ligated into the two restriction sites NdeI and HinIII. The transformed cells were screened for agarose plates containing ampicillin (100 μg/ml), and 10 clones were picked out. The expression construct was screened by double digestion with NdeI and HinIII to confirm the presence of the cDNA insert. The expression construct was propagated in E. coli strain NM522, which lacks the gene for T7 RNA polymerase, was transformed with 10 μl of ligation mixture. The transformed cells were spread on agarose plates containing ampicillin (100 μg/ml) and 10 clones were picked out. The expression construct was screened by double digestion with NdeI and HinIII to confirm the presence of the cDNA insert. The expression construct was propagated in E. coli strain NM522. The nucleotide sequence of the aα or bβ cDNA in the construct was determined by Sanger sequencing; a fluorescence-tagged dideoxy termination method was used with an ABI Automatic Sequencing System (Perkin-Elmer-Cetus) and poly(dA)•poly(dT) primer

Epidermal growth factor (EGF)-IEF—IEF was performed on the precast IsoGel agarose IEF gels (pH range, 3–10; FMC BioProducts, Rockland, ME) under either native or denatured conditions. Gels were stained with Coomassie Brilliant Blue R-250. CD Measurements—CD spectra were measured with an Aviv Circular Dichroism Spectrometer (model 60 DS) (19). The reported CD spectra are the average of 5–10 scans, smoothed by polynomial curve fitting. The fit was checked with a statistical test so that the original data were not oversmoothed. CD data were expressed as molar ellipticity in degrees cm²/dmol, with 115 as residue molecular weight. Fluorescence Measurements—Fluorescence measurements were performed with a Shimadzu spectrofluorometer (model RF-5301PC). Trp fluorescence was measured with an excitation wavelength at 285 nm. The excitation probe, ANS, was used to study the hydrophobicity of protein molecules (20). The ANS (50 μg/ml) in-crystallin (0.1 mg/ml in 0.05 M phosphate buffer, pH 7.6) was incubated at room temperature for 30 min before the measurements were made. The emission was measured with an excitation wavelength at 395 nm.

Chaperone-like Activity Measurements—Chaperone activity was assayed by aggregation of insulin. Aggregation was induced by dithiothreitol reduction of insulin B-chain in the presence and absence of aα-crystallin. Mixture procedures were similar to those reported by Farahbakhsh et al. (21), except that samples were prepared in a 96-well microplate. In brief, 0.4 mg of insulin was reduced with 10 μl dithiothreitol in the presence or absence of aα-crystallin samples (0.05–1.6 μg) in a final volume of 250 μl. The microplate was read at 490 nm with a Bio-Tek EL-800 microplate reader at room temperature. The reading was recorded every 3 min until a plateau was reached. Observed optical densities were recorded and analyzed with the KC3 software provided by Bio-Tek.

Protein concentrations were determined with a Pierce bicinchoninic acid (BCA) assay (22).

RESULTS

Isolation and Cloning of αα- and ββ-Crystallin cDNAs—αα- and ββ-crystallin cDNAs amplified by PCR were analyzed on 2% agarose gel; αα cDNA (689 bp) showed a 0.7-kb band and ββ cDNA (657 bp) showed a 0.65-kb band. After cloning into pAED4 and double digestion with NdeI and HindIII, the 1% agarose gel displayed a 3.3-kb band (3322-bp vector) and a 0.68-kb band (670-bp αα cDNA insert). The expression construct was propagated in E. coli strain NM522. The nucleotide sequence of the aα or bβ cDNA in the construct was determined by Sanger sequencing; a fluorescence-tagged dideoxy termination method was used with an ABI Automatic Sequencing System (Perkin-Elmer-Cetus) and poly(dA)•poly(dT) primer

Expression and Purification of αα- and ββ-Crystallins—For the over-expression of αα- and ββ-crystallins, E. coli strain BL21(DE3) was transformed with expression constructs pAED4-αα and PaeA4-ββ. A colony was picked out from the freshly streaked plate and inoculated with 50 μl of LB containing ampicillin (50 μg/ml). The culture was grown to log phase (A600, 0.6–0.9), a 20-ml volume of the log phase culture was inoculated into 1 liter of LB containing ampicillin (50 μg/ml). The culture was incubated at 37°C with vigorous shaking until the optical density reached 0.6–0.9 at 590 nm, at which point expression was induced by the addition of 1 ml of 1 M isopropyl β-d-thiogalactoside. Incubation was continued for another 3 h. Cells were then harvested by centrifugation at 5,000 rpm for 10 min and kept at −80°C. A control culture of E. coli BL21(DE3) cells with pAED4 was incubated and induced simultaneously.

The collected cells were suspended in TNE buffer (50 mM Tris-HCl, 1 mM EDTA, 100 mM NaCl) at a ratio of 3 ml of buffer to 1 g (wet weight) of cells, and 8 μl of 50 mM phenylmethylsulfonyl fluoride and 80 μl of lysozyme (10 mg/ml) per g of cells were added. The mixtures were kept at 4°C for 20 min, after which deoxycholic acid (4 mg/g of cells) was added. After thorough mixing, DNase (1 mg/ml, 20 μg of E. coli cells) was added, and incubation at 37°C was continued until the mixture was no longer viscous. By centrifugation at 12,000 rpm for 30 min, the cell lysates were separated into supernatant and insoluble pellets. The recombinant proteins were purified from the supernatant by the following successive steps: fractionation with 30–60% saturated ammonium sulfate, gel filtration on Sephacryl S-300HR, and ion exchange chromatography on DEAE-Sephalac. The purity of the recombinant αα- and ββ-crystallins was confirmed with FPLC gel filtration, SDS-PAGE, and isoelectric focusing (IEF) gel electrophoresis, and the crystallin preparations were stored in 0.05 M phosphate buffer at −20°C.

FPLC Gel Filtration—FPLC was carried out on a Superose-6 column (Pharmacia Biotech Inc. FPLC System) (17). The sample solutions were passed through a filter (pore size, 0.45 μm) before application to the column. The column was eluted at 0.5 ml/min. Gel filtration standards from Pharmacia were used to calibrate the column. SDS-PAGE and Western Blot—SDS-PAGE was performed in a slab gel (15% acrylamide) by the method of Laemmli (18) under reducing conditions. Electrophoresis was conducted with a constant voltage of 200 V for 1 h. The gels were stained with Coomassie Brilliant Blue R-250.

Proteins separated by SDS-PAGE were electrophoretically transferred to a nitrocellulose membrane with the Trans-Blot Transfer Cell (Bio-Rad). Transfer was carried out with low field current (30 V and 0.01 A) overnight. The detailed procedures were supplied by the manufacturer (Bio-Rad).

IEF—IEF was performed on the precast IsoGel agarose IEF gels (pH range, 3–10; FMC BioProducts, Rockland, ME) under either native or denatured conditions. Gels were stained with Coomassie Brilliant Blue R-250.
Recombinant Human Lens αA- and αB-Crystallin

CD Measurements—The near-UV CD spectra of recombinant αA- and αB-crystallins are very different (Fig. 5). The difference may not arise solely from the different content of aromatic amino acids (Trp, Tyr, and Phe) but probably also involves a different tertiary structure; CD intensity is related more to the rotational strength of these residues than to their amount, and the rotational strength in turn depends on the compactness of the protein structure (24).

The far-UV CD spectra are shown in Fig. 6; αB-crystallin displays a CD quite different from that of αA-crystallin. For αA-crystallin, the typical 218-nm band for β-sheet conformation is apparent, and the position is shifted to a shorter wavelength for αB-crystallin. For a more precise estimate of the percentage of various secondary structures, we used the computer program SELCON provided by Greenfield (25), which is based on the work of Johnson (26) and Sreerama and Woody (27). The program utilized CD spectra of proteins for which the secondary structures had been determined by x-ray diffraction. Three structural components (α-helix, β-sheet, and β-turn) were obtained, with the remaining structure considered a random coil. The percentage of each secondary structure in this order is 5, 40, 16, and 39% for αA-crystallin and 14, 33, 18, and 35% for αB-crystallin. The figures agree well with previous reports that the major secondary structure of α-crystallin is β-conformation (50–60%, β-sheet and β-turn combined) and that there is very little α-helical content (~5%) (28, 29); however, the present study indicates that αB-crystallin has a greater content of α-helix than αA-crystallin does.

Trp and ANS Fluorescence—The Trp emission maximum provides a direct indication of whether the Trp residues (Trp-9 in αA-crystallin and Trp-9 and Trp-60 in αB-crystallin) are buried or exposed. Since all of these residues are all in the N-terminal domain (residues 1–63), which is hydrophobic and participates in high molecular weight aggregation, they should be relatively buried. αA- and αB-crystallin display emission maxima at 335 and 337 nm, respectively, which are close to that for calf α-crystallin (336 nm) (Fig. 7). ANS is a hydrophobic probe. Upon binding to protein hydrophobic surfaces, ANS fluorescence intensity increases, and the emission maximum shifts to a shorter wavelength, thus providing a sensitive measurement for probing protein hydrophobicity. Fig. 8 shows that ANS fluorescence is more than 3 times greater in αB- than in αA-crystallin, a difference indicating that αB- is more hydrophobic than αA-crystallin.

Chaperone-like Activity—The protection of insulin from aggregation by calf α-crystallin has been extensively studied (21). We included calf α-crystallin for comparison in our study of recombinant αA and αB (Fig. 9). Clearly, αB-crystallin is far more effective than αA-crystallin in preventing insulin aggregation. In the protocol described under “Materials and Methods,” 0.2 mg of αB-crystallin can completely protect insulin from aggregation, whereas 1.6 mg of αA-crystallin is required. Chaperone-like activity thus appears to correlate directly with protein hydrophobicity.

DISCUSSION

Employing the DNA cloning technology, we have prepared pure αA- and αB-crystallins. The aggregation sizes of the pure preparations are slightly smaller than that of α-crystallin isolated from calf lens water-soluble fraction. It is not known whether this difference is due to the lack of modifications during folding and aggregation in E. coli, but α-crystallin does increase in size with development and aging. Conformational studies indicate that αA- and αB-crystallins have an amount of β-conformation comparable with that of calf lens α-crystallin but that αB-crystallin has substantially more α-helical content, whose significance is not clear. The difference in tertiary struc-

![Fig. 1. SDS-PAGE of α-crystallins. Lane 1, standards; lane 2, crude recombinant αA-crystallin; lane 3, purified recombinant αA-crystallin; lane 4, crude recombinant αB-crystallin; lane 5, purified recombinant αB-crystallin; lane 6, human lens α-crystallin; lane 7, calf lens α-crystallin.](Image)

![Fig. 2. IEF of α-crystallins in native (A) and denatured (B) conditions. Lane 1, markers; lane 2, recombinant αA-crystallin; lane 3, recombinant αB-crystallin; lane 4, human lens α-crystallin.](Image)
ture, as manifested by the difference in near-UV CD (Fig. 5), may contribute to conformational stability. To our knowledge, no study of the difference in conformational stability between recombinant αA- and αB-crystallin has been reported.

The most striking difference between αA- and αB-crystallin is the greater hydrophobicity and thus the greater chaperone-like activity of αB-crystallin. The significance of this finding is related to the facts that αB-crystallin is expressed in more tissues than αA-crystallin (13), that αB-crystallin is expressed at higher levels than αA-crystallin in some tissues (30, 31), and that αB-crystallin, like the small heat-shock protein, may function as a stress protein and may be expressed under various stress conditions and diseases (32–34). The greater chaperone function of αB-crystallin thus effectively stabilizes other proteins under stress and confers thermostability to cells. The mechanism for the chaperone-like activity of α-crystallin is interaction with other proteins partially unfolded by heat, denaturant, or other mechanisms such as the reduction of insulin (21, 35, 36).

The recombinant αA- and αB-crystallins thus provide the advantage that they are in the native form, which can be important to studies of the dynamic quaternary structure of α-crystallin. Previous studies with renatured α-crystallin (ho-
mopolymer and heteropolymer) indicated that the αA and αB subunits are structurally equivalent and interchangeable (23, 39). Such studies using recombinant αA- and αB-crystallins may yield more relevant data, since in vivo αA and αB subunits are interchanged under the native conditions after αA- and αB-crystallins are expressed and folded.

The biological significance of the 3:1 ratio of αA to αB sub-units in the mammalian α-crystallin has not been established, although previous reports indicated that any ratios can be formed in vitro (23, 39). It is believed that the significance of the αA:αB ratio may be implied by the result of a comparative study on the conformational stability of α-crystallins formed by various ratios of αA and αB subunits. It is possible that α-crys-

![Fig. 6. Far-UV CD spectra of recombinant α-crystallins. The protein concentration is 1.5 mg/ml, and the cell path length is 0.5 mm. The CD ellipticity units are degrees cm²/dmol.](image)

![Fig. 7. Trp fluorescence of recombinant α-crystallins. The excitation wavelength is 295 nm, and the protein concentration is 0.1 mg/ml.](image)

![Fig. 8. ANS fluorescence of recombinant α-crystallins. The excitation wavelength is 395 nm. The protein and ANS concentrations are 0.1 mg/ml and 50 μM, respectively.](image)

![Fig. 9. Chaperone-like activity of α-crystallins. The insulin and dithiothreitol concentrations are 0.4 mg/ml and 10 mM, respectively. The numbers to the right of curves are the weight ratios of insulin to α-crystallin (mg/mg).](image)
tallin with a 3:1 ratio of αA to αB is the most stable form.

A recent chaperone study used a site-specific mutation on αA-crystallin to study conformational change of the substrate bound to α-crystallin (40). A Trp-free αA-crystallin mutant was prepared, and Trp fluorescence of the substrate protein was probed during the chaperone action. The results, along with those of many previous recombinant studies (4–11), demonstrated the versatility of the recombinant DNA technology. During cataract formation, many posttranslational modifications, including nonenzymatic glycation, protein-protein and mixed disulfide formation, and photooxidation of Trp residues, were observed. Use of site-specific α-crystallin mutants may help us to understand modification-induced aggregation and insolubilization. For example, use of a Trp-free α-crystallin mutant in the UV irradiation studies may shed light on the role of Trp residues in lens damage due to sunlight. This and other studies mentioned above are under way in our laboratory.

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REFERENCES

1. Harding, J. (1991) Cataract: Biochemistry, Epidemiology and Pharmacology, pp. 1–70, Chapman & Hall, London
2. Spector, A. (1984) Invest. Ophthalmol. & Visual Sci. 25, 130–146
3. Datiles, M. B., Schumer, D. J., Zigler, J. S., Jr., Russell, P., Anderson, L., and Garland, D. (1992) Curr. Eye Res. 11, 669–677
4. Dubin, R. A., Wawrousek, E. F., and Piatigorsky, J. (1989) Mol. Cell. Biol. 9, 1083–1091
5. Merck, K. B., de Haard-Hoekman, W. A., Oude Essink, B. B., Bloemendal, H., and de Jong, W. W. (1992) Biochim. Biophys. Acta 1130, 267–276
6. van den Heuvel, P. R. L. A., Overkamp, P., Knauf, U., Gaestel, M., and de Jong, W. W. (1994) FEBS Lett. 355, 54–58
7. Smulders, R. H. P. H., Merck, K. B., Arnedokerk, J., Horwitz, J., Takemoto, L., Slingsby, C., Bloemendal, H., and de Jong, W. W. (1995) Eur. J. Biochem. 232, 834–838
8. Smulders, R. H. P. H., van Geel, I. G., Gerards, W. L. H., Bloemendal, H., and de Jong, W. W. (1995) J. Biol. Chem. 270, 13916–13924
9. Plater, M. L., Goode, D., and Crabbe, M. J. C. (1996) J. Biol. Chem. 271, 26558–26566
10. Smulders, R. H. P. H., Carver, J. A., Lindner, R. A., van Boeckel M. A. M., Bloemendal, H., and de Jong, W. W. (1996) J. Biol. Chem. 271 29060–29066
11. Andley, U. P., Mathur, S., Griest, T. A., and Petrais, J. M. (1996) J. Biol. Chem. 271 31973–31980
12. Bhat, S. P., Horwitz, J., Srivivasan, A., and Ding, L. (1991) Eur. J. Biochem. 202, 775–781
13. Sax, C. M., and Piatigorsky, J. (1994) Adv. Enzymol. 69, 155–201
14. Deretic, D., Aebersold, R. H., Morrison, H. D., and Papermaster, D. S. (1994) J. Biol. Chem. 269, 16853–16861
15. Iwaki, T., Iwaki, A., Liem, R. K., and Goldman, J. E. (1991) Kidney Int. 40, 52–56
16. Jin, J.-P. (1995) J. Biol. Chem. 270, 6908–6916
17. Liang, J. N., and Li, X-Y. (1997) Nature 227, 680–685
18. Liang, J. N., and Rossi, M. (1989) Invest. Ophthalmol. & Visual Sci. 30, 2065–2068
19. Johnson, J. D., El-Bayoumi, M. A., Weber, L. D., and Tulinsky, A. (1979) Biochemistry 18, 1292–1296
20. Farahbakhsh, Z. T., Huang, Q-L., Ding, L-L., Altenbach, C., Steinhoff, H-J., Horwitz, J., and Hubbell, W. L. (1995) Biochemistry 34, 509–516
21. Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J., and Kieser, D. C. (1986) Anal. Biochem. 150, 76–80
22. Thomson, J. A., and Augusteiny, R. C. (1989) Biochm. Biophys. Acts 994, 246–252
24. Cantor, C. R., and Timasheff, S. N. (1982) Proteins, Vol. V, pp. 145–306, Academic Press, Inc., New York
25. Greenfield, N. J. (1996) Anal. Biochem. 235, 1–10
26. Johnson, W. C., Jr. (1990) Proteins Structure Funct. Genet. 7, 205–214
27. Sreerama, N., and Woody, R. W. (1994) J. Mol. Biol. 242, 497–507
28. Liang, J. N., and Charakabarti, B. (1982) Biochemistry 21, 1847–1852
29. Liang, J. N., Andley, U. P., and Chylack, L. T., Jr. (1985) Biochim. Biophys. Acts 832, 197–203
30. Iwaki, T., Iwaki, A., and Goldman, J. E. (1993) Acta Neuropathol. 85, 475–480
31. Kato, K., Shinohara, H., Kurube, N., Inaguma, Y., Shimizu, K., and Ohshima, K. (1991) Biochim. Biophys. Acts 1074, 201–208
32. van Noort, J. M., van Sechel, A. C., Bajramovic, J. J., ElOuagmiri, M., Polman, C. H., Lassmann, H., and Ravid, R. (1995) Nature 375, 798–801
33. Iwaki, T., Iwaki, A., Tateishi, J., Sakaki, Y., and Goldman, J. E. (1993) Am. J. Pathol. 143, 487–495
34. Hitotsumatsu, T., Iwaki, T., Fukui, M., and Tateishi, J. (1996) Cancer 77, 352–361
35. Horwitz, J. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 10449–10453
36. Wang, K., and Spector, A. (1994) J. Biol. Chem. 269, 13601–13608
37. Wang, K., Ma, W., and Spector, A. (1995) Exp. Eye Res. 61, 115–124
38. Li, L.-K., and Spector, A. (1973)
39. vandenOetelaar, P. J., van Someren, P. F., Thomson, J. A., Siezen, R. J., and Hoenders, H. J. (1990) Biochemistry 29, 3488–3493
40. Das, K. P., Petras, J. M., and Surewicz, W. K. (1996) J. Biol. Chem. 271, 10449–10452