Amyloid Fibril Formation by Lens Crystallin Proteins and Its Implications for Cataract Formation

Received for publication, July 28, 2003, and in revised form, October 17, 2003
Published, JBC Papers in Press, November 13, 2003, DOI 10.1074/jbc.M308203200

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The α-, β-, and γ-crystallins are the major structural proteins within the eye lens and are responsible for its exceptional stability and transparency. Under mildly denaturing conditions, all three types of bovine crystallin assemble into fibrillar structures in vitro. Characterization by transmission electron microscopy, dye binding assays, and x-ray fiber diffraction shows that these species have all of the characteristics of fibrils associated with the family of amyloid diseases. Moreover, the full-length proteins are incorporated into the fibrils, (i.e., no protein cleavage is required for these species to form), although for the γ-crystallins some fragmentation occurs under the conditions employed in this study. Our findings indicate that the inherent stability of the β-sheet supramolecular structure adopted by the crystallins in the eye lens and the chaperone ability of α-crystallin must be crucial for preventing fibril formation in vivo. The crystallins are very stable proteins but undergo extensive post-translational modification with age that leads to their destabilization. The ability of the crystallins to convert into fibrils under destabilizing conditions suggests that this process could contribute to the development of cataract with aging.

The healthy eye lens is transparent and serves to focus light on to the retina. The predominant proteins within the lens are the crystallins, and lens transparency is thought to be maintained by a liquid-like, short range order present in highly concentrated solutions of these proteins (1). The high refractive index in the lens is aided by the absence of cellular organelles in the post-mitotic lens fiber cells. With the exception of the outer epithelial layer, there is almost no protein turnover in the lens and therefore the crystallins must be maintained in a stable structure throughout the lifespan of the organism (1).

The crystallins are highly stable proteins that are organized in a supramolecular β-sheet structure within the lens (1). In mammals, there are three classes of crystallins denoted α, β, and γ, each of which has a variety of distinct subunits. α-Crystallin, the principal lens protein, is a member of the small heat shock protein family and acts as a molecular chaperone (2). In its chaperone role, α-crystallin has been shown to inhibit aberrant aggregation and insolubilization of a wide range of proteins under in vitro stress conditions generated, for example, by elevated temperature, chemical reduction, or oxidation (2, 3).

α-Crystallin consists of two closely related subunits, αA and αB. αB-Crystallin is found in significant quantities in a variety of locations outside the eye lens where it is stress-inducible (3–8). Its overexpression is associated with a diversity of disease states including conditions associated with amyloid fibril deposition such as Alzheimer’s, Creutzfeldt-Jakob, and Parkinson’s diseases (2–7). Both αA- and αB-crystallins are present in the lens where one of their likely roles is to chaperone all of the crystallin proteins including themselves (9), thus preventing extensive protein aggregation and precipitation. The α-crystallin complex is a highly heterogeneous aggregate of 20-kDa subunits, giving rise to multimers in the mass range of ~300–1000 kDa. The β-crystallins are smaller aggregates comprising ~20–30 kDa subunits, giving complexes of ~50–200 kDa, whereas the γ-crystallins are found as monomers of ~20 kDa in mass (1). The β-crystallin subunits are structurally related to the γ-crystallins, and crystal structures are available for representative β- and γ-crystallin subunits (10). No structure has yet been determined for the α-crystallin subunits.

The combination of highly stable, long-lived proteins and a high concentration of molecular chaperones implies that the enclosed lens is well equipped to deal with any misfolding of its component proteins. However, the occurrence of cataract, which arises from crystallin aggregation and precipitation, reveals that there are, nonetheless, shortcomings with respect to lens protein stability. Cataract is defined as opacity of the eye lens and is the leading cause of vision impairment worldwide and is responsible for ~40–80% of the estimated 45 million cases of blindness that occur across the globe (11, 12). Despite the widespread nature of this disease, the only treatment available at present is surgical removal of the lens and replacement with a plastic intraocular lens (1, 11). A better understanding of the molecular mechanisms of cataract formation may aid the development of a therapeutic strategy that could avoid the need for surgery.

Two possible mechanisms, which are not mutually exclusive, may cause cataract. The first is a condensation phenomenon, whereby opacity results from loss of solubility of the crystallins.

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For example, the point mutation R14C in human γD-crystallin causes a progressive juvenile-onset hereditary cataract and there is evidence that oxidation of R14C γD-crystallin leads to the formation of disulfide-linked oligomers, which eventually precipitate (13). In other cases, the loss of lens clarity is believed to result from spontaneous crystallization of mutant γD-crystallins (14). These mutations dramatically lower the solubility of the protein and increase the crystal nucleation rate relative to that of the wild-type protein.

In the second mechanism, cataract is seen as a conformational disorder (15, 16) where unfolding or destabilization of the crystallin proteins drives cataractogenesis. For example, in the case of T5P γ-crystallin, a mutant associated with a number of phenotypes including Coppel-like cataract, there appears to be a direct causal relationship between cataract and partial destabilization and unfolding of the mutant crystallin protein (17). In another example of a conformational disorder, amyloid deposition by a βnop-crystallin mutant appears to be a cause of a murine cataract (18). Evidence for the latter conclusion includes identification of intranuclear filamentous inclusions in vivo that stain with the amyloid-detecting dye Congo Red and the ready assembly of the mutant βnop-crystallin into amyloid fibrils in vitro under conditions where the wild-type protein remains soluble.

Formation of amyloid deposits in the eye lens would potentially disturb the short range order of the crystallins and thus lead to lens opacity and cataract. In this paper, we report that all three classes of wild-type bovine crystallin proteins, including the molecular chaperone α-crystallin, are capable of forming amyloid fibrils when subject to unfolding conditions in vitro. We show that these fibrils have the characteristic amyloid structure by transmission electron microscopy (TEM), dye binding assays, and x-ray fiber diffraction. The inherent ability of the crystallins to convert into fibrils suggests that this process could contribute to the development of cataract with aging and indicates that the environment in the healthy eye lens must inhibit this process under normal circumstances.

EXPERIMENTAL PROCEDURES

Purification of α, β, and γ-Crystallin—Lenses were extracted from calf eyes obtained from an abattoir (Wollondilly Abattoirs Pty Ltd, Picton, New South Wales 2571, Australia) and stored at −20 °C. Crystallin proteins were purified from bovine lenses as described previously by standard methods via size exclusion chromatography from cows of less than two years in age (19). SDS-polyacrylamide gel electrophoresis was used to confirm the purity of the α-, β-, and γ-crystallins.

Formation of Amyloid Fibris by Bovine α-Crystallin at pH 7.4—Bovine α-crystallin was dissolved at 10 mg/ml in 0.1 M phosphate buffer, pH 7.4, and incubated at 60 °C for 24 h with varying concentrations of guanidine hydrochloride (GdnHCl) (0.001–1 M).

Formation of Amyloid Fibris by Bovine α-Crystallin at pH 2.0—α-Crystallin was dissolved at 10 mg/ml in 10% (v/v) trifluoroethanol (TFE), adjusted to pH 2.0 with HCl, and incubated at 60 °C for 4 h.

Formation of Amyloid Fibris by Bovine β-Crystallin and γ-Crystallin at pH 2.0—β-Crystallin was dissolved at 10 mg/ml in 10% (v/v) TFE, adjusted to pH 2.0 with HCl, and incubated at room temperature for 10 h. γ-Crystallin was dissolved at 10 mg/ml in 10% (v/v) TFE, adjusted to pH 2.0 with HCl, and incubated at room temperature for 1–3 weeks. Fibril formation was accelerated by elevating the temperature such that the β- and γ-crystallins formed fibrils after incubation for 3 h at 60 °C.

TEM—Formvar and carbon-coated nickel electron microscopy grids were prepared by the addition of 5 μl of protein sample at a concentration of 1 mg/ml. The grids were then washed with 3 × 10 μl H2O and negatively stained with 10 μl of uranyl acetate (2% w/v), (Agar Scientific). The grids were dried with filter paper between each step. Samples were viewed under 20–125-K magnifications at 120-kV excitation voltages using a Philips Tecnai transmission electron microscope, and images were analyzed by a MegaView II Soft imaging system.

Congo Red Assays—Fresh solutions of Congo Red (CR) (20) in 5 mM potassium phosphate buffer containing 150 mM NaCl, pH 7.4, were passed through a 0.2-μm filter immediately before use. The CR solutions were added to 100 μg/ml protein solutions to a final dye concentration of 0.5 μM, and the samples vortexted for 15 s. The absorption spectrum of each sample was recorded from 400 to 700 nm on a Varian Cary Bio UV-visible spectrophotometer using 1-cm path length quartz cuvettes and corrected for contributions from buffer and protein. The spectrum of CR alone was compared with that of CR solutions in the presence of protein. A red shift of the absorption band toward 540 nm and an increase in absorption were together taken to be indicative of the formation of amyloid structures.

Thioflavin T Assay—The spectrum of thioflavin T (ThT) alone was compared with that of protein solutions (0.05 mg/ml) containing ThT at a final dye concentration of 50 μM in 10 mM phosphate, 150 mM NaCl, adjusted to pH 7.0. An increase in the fluorescence emission intensity at 490 nm was taken to be indicative of amyloid formation (21). Fresh solutions of ThT were passed through a 0.2-μm filter immediately before use. A Varian Cary Eclipse fluorescence spectrophotometer was used to record the spectra with 1-cm path length cuvettes (λex = 442 nm; λem = 490–550 nm).

Fibril Purification—Pepsin was added to an α-crystallin fibril solution (0.001% w/v pepsin: α-crystallin) and incubated at 37 °C for 1 h (22). The sample was then ultra centrifuged at 90,000 rpm for 2 h in a Beckman Coulter Optima TL-X ultracentrifuging system using a TLA 100 rotor. The supernatant was subsequently carefully removed, and the fibril pellet was re-suspended in minimal buffer.

x-ray Fiber Diffraction—α-Crystallin samples for x-ray fiber diffraction were prepared by air-drying a 12-μl aliquot of concentrated pepsin-purified fibril solution between two wax-filled capillary ends. The capillaries were separated slowly while drying using a stretch frame apparatus to enhance alignment of the fibrils. A small stalk of fibrils protruding from the end of one of the capillaries was obtained. The sample was aligned in an x-ray beam, and diffraction images were recorded using a Rigaku D/Max–R rotating anode x-ray source (wavelength, 1.5418 Å) and an R-AXIS IV image plate x-ray detector. Images were analyzed by Java application “Crystal Clear” software. Samples for x-ray diffraction from β-crystallin and γ-crystallin were prepared directly from 12-μl fibril stocks (10 mg/ml) without further pepsin purification.

Fibril Solubilization—Fibril samples were centrifuged at 90,000 rpm in a Beckman Coulter Optima TL-X ultracentrifuging system using a TLA 100 rotor for 2 h. The supernatant was then carefully removed from the pellet. The fibril pellet was resuspended in 90% (v/v) acetonitrile to ~10 mg/ml and incubated at room temperature for 24 h. TEM analysis showed clearly that no detectable quantities of fibrils remained after this treatment.

SDS-PAGE—Fibrils, pre-fibrillar protein, and the supernatants of the crystallin samples were analyzed by SDS-PAGE to investigate whether low molecular weight fragments were present. First, fibrils were solubilized according to the method described above. Protein samples were separated on a 12% NuPAGE bis-tris gel with NuPAGE MOPS buffer over 60 min at 200 V and stained with NuPAGE Simply Blue safestain. Novex Mark 12 wide-range protein standards were used for mass calibration (Invitrogen).

RESULTS

Fibril Formation by α-Crystallin at Neutral pH—α-Crystallin was incubated at 10 mg/ml in 0.1 M potassium phosphate buffer, pH 7.4, with varying concentrations (0.001–1.0 μl) of GdnHCl as denaturant. After incubation for 24 h at 60 °C, each sample was examined by TEM. In very low concentrations of GdnHCl (0.001 μl), large, roughly spherical aggregates of α-crystallin were present (Fig. 1A) with diameters of 7–15 nm. In 0.1 μl GdnHCl, large asymmetric aggregates and chainlike structures with the appearance of a row of beads (Fig. 1B) were observed. The individual beads had a diameter similar to that observed for the similar structures formed in the presence of very low concentrations of GdnHCl (i.e. 7–15 nm). In higher concentrations of GdnHCl (0.75–1.0 μl) (Fig. 1C and D), a mixture of structures was observed by TEM including long smooth fibrils in addition to the spherical aggregates and rows

1 The abbreviations used are: TEM, transmission electron microscopy; Ajf, amyloid β; GdnHCl, guanidine hydrochloride; TFE, trifluoroethanol; ThT, thioflavin T; CR, Congo Red; MOPS, 4-morpholinepropanesulfonic acid.
of beads observed previously (Fig. 1D). The α-crystallin fibrils formed in 1 M GdnHCl were ~10 nm in diameter and 50–400 nm in length (Fig. 1, C and D) and were very similar in morphology to fibrils formed by amyloidogenic proteins such as β2-microglobulin at acidic pH (23). Although the aggregates formed at low concentrations of GdnHCl at 60 °C bound the amyloid-detecting dye ThT, the degree of binding was less than that induced by the presence of the fibrils formed in high concentrations of GdnHCl (Fig. 1, E). The insert in Fig. 1E highlights the overall change in ThT binding for these solutions.

The TEM images of heated α-crystallin in the presence of low concentrations of GdnHCl (Fig. 1A) show structures of similar size to those observed previously for α-crystallin upon heat incubation in the absence of denaturant (24). The bead-like structures, emerging at higher GdnHCl concentrations (0.1 M) at elevated temperatures, arise from what appears to be attractive interactions between spherical α-crystallin aggregates, which may result from an increased exposure of hydrophobic residues in the presence of denaturant. The rows of beads are similar in appearance to the aggregates that form from native α-crystallin in the absence of denaturant at temperatures above 45 °C (24–28). Burgio et al. (28) reported a tendency for these beadlike structures to transform into fibrillar structures at higher temperatures (~70 °C), although the length of these fibrillar structures was significantly shorter (<100 nm) than the fibrils observed in this study (Fig. 1, C and D). We hypothesize that the formation of smooth long fibrils at physiological pH results from increasingly denaturing conditions at higher concentrations of GdnHCl and elevated temperatures, which cause partial unfolding of the α-crystallin subunits (29–31) and give rise to conditions conducive to fibril formation. Indeed, we observed that at room temperature in the presence of 1 M GdnHCl, only amorphous aggregates of α-crystallin were observed over a 24-h time period (data not shown). Hence, although 1 M GdnHCl is likely to induce partial unfolding followed by self-assembly of α-crystallin, elevated temperatures are required to generate fibril assembly within 24 h. A similar phenomenon is observed for the yeast prion protein Ure2p where conversion from fibrils composed of a polymeric assembly of native-like helical Ure2p subunits into amyloid fibrils is promoted by heat stress (32, 33).

In contrast to α-crystallin, β- and γ-crystallins aggregated and formed amorphous precipitates after <1 h under these conditions (i.e. 10 mg/ml, 1 M GdnHCl, 60 °C, 0.1 M phosphate, pH 7.4). The tendency of β- and γ-crystallins to precipitate
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Fig. 2. Transmission electron micrographs of bovine crystallins. The crystallins were suspended at 10 mg/ml in 10% (v/v) TFE, 90% (v/v) H2O adjusted to pH 2.0 with HCl. A, α-crystallin immediately after dissolution. B, α-crystallin after 4-h incubation at 60 °C. C, short straight fibrils. D, β-crystallin immediately after dissolution. E, γ-crystallin immediately after dissolution. F, γ-crystallin after 1 week of incubation at room temperature. Protein samples were diluted to 1 mg/ml immediately prior to examination by electron microscopy.

when subjected to destabilizing conditions (e.g. temperatures of about 60 °C) is well recognized and is much greater than that of the molecular chaperone, α-crystallin. In fact, a common method of assessing the chaperone ability of α-crystallin, which does not precipitate upon heating to these temperatures, is to heat a mixture of the two proteins together and monitor the inhibition by α-crystallin of precipitation of β- or γ-crystallin (2).

Fibril Formation by the Crystallin Proteins at Acidic pH—We next subjected the bovine crystallins to acidic pH and assessed their propensity to form amyloid fibrils under these conditions. All three classes of bovine crystallins, α-, β-, and γ, were dissolved at 10 mg/ml in 10% (v/v) TFE in H2O at pH 2.0. TFE destabilizes the native structure of α-crystallin (34), and low concentrations of TFE (10–20% v/v) increase the rate of fibril formation by the amyloid β (Aβ) peptide (35). Amyloid fibril formation by other proteins is often induced in vitro by denaturation under acidic conditions (see Refs. 36 and 37). TEM showed clearly that all of the crystallins underwent major structural changes under these conditions. Thus, immediately after dissolving α-crystallin under these conditions, aggregates of average diameter ~15–30 nm were observed by TEM (Fig. 2A), which are typical of native α-crystallin. These aggregates significantly increased the fluorescence of ThT (Fig. 3). After incubation at 60 °C for 24 h, however, fibrils were observed by TEM (Fig. 2B) and concomitantly a further increase in the fluorescence emission intensity of ThT occurred (Fig. 3). These fibrils have an average diameter of 15 nm and a length in the order of one to a few microns. There appears to be two types of α-crystallin fibril morphology, remarkably similar to the amyloid fibrils that form from the Aβ-peptide in 20% (v/v) TFE (35), i.e. long straight fibrils (Fig. 2B, △) and short curly elongated aggregates (Fig. 2B, ▲). It is possible that that the short curly

aggregates of α-crystallin were a linear association of native subunits or that they were precursors to amyloid fibrils (‘proteofibrils’). The long straight fibrils (Fig. 2B, △) have the dimensions and a morphology clearly similar to classic amyloid fibrils formed by a wide range of peptides and proteins (38).

β-Crystallin and γ-crystallin also formed fibrils under these solution conditions (i.e. 10 mg/ml, in 10% (v/v) TFE, pH 2.0) (Fig. 2, D and F). Following dissolution of the protein, monomeric subunits and aggregates were observed by TEM (Fig. 2, C and E) for both proteins, similar to α-crystallin. After incubation of β-crystallin for 10 h at room temperature or of γ-crystallin for 1 week at room temperature, fibril formation was evident from the TEM images (Fig. 2, D and F). As for α-crystallin, there was a relative increase in ThT fluorescence emission intensity (Fig. 3) between the pre-fibrillar (Fig. 2, C and E) and fibrillar (Fig. 2, D and F) aggregates. These fibrils of β-crystallin and γ-crystallin have an average diameter of ~11 nm. Unlike α-crystallin, heating to high temperature (60 °C) was not required for fibril formation by either β- or γ-crystallin, indicating that β- and γ-crystallins form fibrils under milder conditions than α-crystallin. However, heating accelerated the formation of β- and γ-crystallin fibrils and well defined fibrils were visible by TEM after just 2–4 h incubation at 60 °C under the same solution conditions (data not shown). Solutions containing 10% (v/v) acetonitrile at pH 2.0 were also effective at inducing fibril formation but on a time scale longer than that required in the presence of TFE, typically 1 week for β- and γ-crystallin when incubated at 60 °C (data not shown).

The absorption spectra of Congo Red on binding to both pre-fibrillar aggregates (Fig. 2, A, C, and E) and fibrils (Fig. 2, B, D, and F) were measured, and the results are summarized in Fig. 4 and Table I. Each type of crystallin bound Congo Red in a 1:1 pattern characteristic of amyloid fibrils (39), i.e. meridional reflections of 4.6–4.7 Å that correspond to the spacing between adjacent hydrogen-bonded β-strands lying perpendicular to the fibril axis and broad equatorial reflections at 9–11

Fig. 3. ThT assay. Crystallins were dissolved to ~10 mg/ml in H2O, 10% (v/v) TFE, adjusted to pH 2.0, and incubated at 60 °C. The average fluorescence emission intensity at 490 nm (λex = 442 nm) was measured for ThT (50 μM) and compared with that of 50 μM ThT containing 0.05 mg/ml protein at t = 0 h and at t = 24 h in 10 mM phosphate buffer, 150 mM NaCl, adjusted to pH 7.0. a.u., arbitrary units.

X-ray fiber diffraction was also employed to characterize the crystallin fibrils formed at pH 2.0. A high proportion of non-fibrillar aggregates is visible in the TEM images of α-crystallin samples (Fig. 1B). Prior to analysis by x-ray fiber diffraction, the α-crystallin fibrils were purified according to the method of Zurdo et al. (22). No detectable quantities of non-fibrillar aggregates were observed in the TEM images of β- and γ-crystallin fibril samples; thus, no further purification was required for these specimens. Each crystallin class exhibited an anisotropic cross-β-pattern characteristic of amyloid fibrils (39), i.e. meridional reflections of 4.6–4.7 Å that correspond to the spacing between adjacent hydrogen-bonded β-strands lying perpendicular to the fibril axis and broad equatorial reflections at 9–11
Fig. 4. CR assay. Crystallins were dissolved to ~10 mg/ml in H2O, 10% (v/v) TFE, adjusted to pH 2.0, and incubated at 60 °C. A, α-crystallin; B, β-crystallin, C, γ-crystallin. The spectrum of CR alone (0.5 mM) was compared with that of CR solutions in the presence of protein (100 mg/ml) at t = 0 h and at t = 4 h in 5 mM potassium phosphate buffer, 150 mM NaCl, adjusted to pH 7.4. Difference spectra are shown, which have been corrected for the contribution from buffer. a.u., arbitrary units.

Fig. 5. X-ray fiber diffraction pattern of α-crystallin fibrils. Images were analyzed by Crystal Clear software, ▲, meridional reflection at 4.7 Å; ○, equatorial reflection at 10.4 Å. See “Experimental Procedures” for sample preparation.

Á that arise from the separation between the β-sheets that run parallel to the fibril axis. Fig. 5 shows the diffraction pattern obtained for α-crystallin fibrils, and the approximate position of the reflection maxima measured for each crystallin class is reported in Table I.

The fibril samples (Fig. 2, B, D, and F) were analyzed by SDS-PAGE to assess whether the proteins had undergone degradation when subjected to the conditions employed to induce fibril formation. SDS-PAGE of solubilized fibrils revealed that neither α-crystallin (Fig. 6A) nor β-crystallin (Fig. 6B) had undergone any detectable degradation during fibril formation. Therefore, it is concluded that the fibrils are composed of full-length protein. γ-Crystallin did, however, undergo partial degradation with both full-length protein and fragmented γ-crystallin being incorporated into the fibrils (Fig. 6C).

DISCUSSION

It has been shown previously that a γBnop-crystallin mutant associated with an early onset murine cataract assembles into amyloid fibrils under physiological conditions in vitro, unlike the wild type protein (18). We have now demonstrated that under increasingly denaturing conditions, all three major classes of wild-type bovine crystallins assemble into amyloid fibrils.

Despite our findings that the crystallins form amyloid fibrils in vitro under destabilizing conditions, neither fibrils nor filaments are observed in normal lenses. Because crystallin aggregation is deleterious to the transparency of the eye lens, it is evident that strict control mechanisms are in place to prevent fibril formation. Two mechanisms are likely to contribute to the long term stability of proteins in the lens. Firstly, the crystallins pack into stable supramolecular assemblies and the inherent stability of these higher order structures is likely to contribute to the structural integrity of the lens (1, 3, 4). This supramolecular assembly of crystallins in the lens fiber cells itself binds Congo Red and ThT dyes (40). Given that amyloid fibrils are part of a larger group of related structures, native crystallins in the lens may have amyloid-like characteristics, although filamentous forms are not visible in healthy lenses.

A second mechanism likely to prevent amyloid fibril assem-
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Degradation of α-, β-, and γ-crystallins from both of their terminal regions occurs in the aged eye lens (e.g. 50–52), and these processes may contribute to cataract formation via structural destabilization and potentially increased fibril-forming propensity. In this study, although γ-crystallin fibrils contain both full-length protein and fragments under conditions used here, we have demonstrated that full-length α- and β-crystallin protein fibrils form fibrils, revealing that degradation is not essential for fibril formation under otherwise destabilizing conditions. The truncation of these proteins observed in vitro is, however, likely to contribute to their destabilization.

A link has recently been reported between supranuclear cataract and Alzheimer’s disease (53). Goldstein et al. (53) identified the Aβ-peptide, the major proteinaceous component of deposits in Alzheimer’s disease, in the eye lens and provided evidence to show that Aβ promoted α-crystallin aggregation and amyloid formation in the lens, thus resulting in cataract. Sandilands et al. (18) have also recently demonstrated a direct link between amyloid fibril formation and cataract. Although amyloid fibrils are associated with pathological symptoms in a diverse range of diseases (54), their specific role in inducing disease remains unclear. In the case of the eye lens, formation of amyloid fibrils is likely to disrupt lens homogeneity, thereby resulting in the scattering of light. Given our observation that full-length crystallin proteins will assemble into amyloid fibrils in vitro under destabilizing conditions, the possibility arises that crystallins will eventually convert into amyloid fibrils in the lens as a result of a number of factors, including the strain of longevity required of them in the lens nucleus while being subject to destabilizing environmental conditions over many years along with the diminished chaperone ability of α-crystallin in old age (4, 19). This effect is likely to be enhanced in the nucleus, the region of lens opacity common in age-related (senile) nuclear cataract, suggesting that this form of cataract could perhaps be associated with amyloid formation.

Acknowledgments—We thank Dr. Robyn Lindner and Professor Roy Quinlan for assistance and advice during the initial phase of this investigation. We thank Dr. J. J. Rickard and Dr. H. Dobberstein (Cavendish Laboratory, University of Cambridge) for assistance with transmission electron microscopy.

REFERENCES

1. Harding, J. J. (1991) Cataract: Biochemistry, Epidemiology, and Pharmacology. Chapman and Hall, London.
2. Horwitz, J. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 10449–10453.
3. Horwitz, J. (2000) Semin. Cell Dev. Biol. 11, 53–60.
4. Derham, B. K., and Harding, J. J. (1999) Prog. Retin. Eye Res. 18, 463–509.
5. van Montfort, R., Slingsby, C., and Vierling, E. (2001) Adv. Protein Chem. 59, 105–156.
6. Horwitz, J. (2003) Exp. Eye Res. 76, 145–153.
7. Treweek, T. M., Morris A. M., and Carver J. A. (2003) Aust. J. Chem. 56, 357–367.
8. Clark, J. I., and Muchowski, P. J. (2000) Curr. Opin. Struct. Biol. 10, 52–59.
9. Sun, T. X., and Liang, J. J. N. (1998) J. Biol. Chem. 273, 286–290.
10. Slingsby, C., and Clout, N. J. (1999) Eye 13, 285–292.
11. Snellingen, T., Evans, J. B., Ravilla, T., and Foster, A. (2002) The Cochrane Library, Issue 4, John Wiley & Sons, Inc., New York.
12. Nakatsu, Y., Otsu, K., and Kanai, A. (2001) Nippon Ganka Gakkai Zasshi 105, 369–373.
13. Pande, A., Pande, J., Asherie, N., Lomakin, A., Ogun, O., King, J. A., Lubsen, N. H., Walton, D., and Benedek, G. B. (2003) Proc. Natl. Acad. Sci. U. S. A. 97, 1993–1998.
14. Pande, A., Pande, J., Asherie, N., Lomakin, A., Ogun, O., King, J., Lubsen, N. H., Walton, D., and Benedek, G. B. (2004) Proc. Natl. Acad. Sci. U. S. A. 96, 6116–6120.
15. Harding, J. J. (1995) Curr. Opin. Ophthalmol. 6, 10–13.
16. Heon, E., Priston, M., Schorderet, D. F., Billingsley, G. D., Girard, P. O., Lubsen, N., and Munner, F. L. (1999) Am. J. Hum. Genet. 65, 1261–1267.
17. Fu, J., and Liang, J. J. N. (2002) FEBS Lett. 513, 213–217.
18. Sandilands, A., Hutcheson, A. M., Lutz, R. B., Graw, J., Masaki, S., Dobson, C. M., MacPhee, C. E., and Quinlan, R. A. (2002) EMBO J. 21, 6505–6514.
19. Carver, J. A., Nichols, K. A., Aquilina, J. A., and Truscott, R. W. (1996) Exp. Eye Res. 63, 639–647.
20. Glimcher, G. G., Eanes, E. D., and Page, D. L. (1972) J. Histochem. Cytochem. 20, 821–826.
21. LeVine, H., III (1999) Methods Enzymol. 309, 274–284.
22. Zardi, J., Guirarro, J. I., and Dobson, C. M. (2001) J. Am. Chem. Soc. 123, 9841–9842.

2 A. Rekas, J. A. Carver, N. Williamson, and R. Cappai, unpublished observations.

bly in the lens resides with the ability of α-crystallin to act as a molecular chaperone, thereby preventing aberrant crystallin protein aggregation. α-Crystallin has been shown to inhibit amyloid formation by apolipoprotein C-II in vitro (41), and αβ-crystallin is known to prevent the formation of amyloid structures by a variety of unrelated peptides and proteins, e.g. the Aβ-peptide (42, 43), α-lactalbumin, and α-synuclein. Therefore, it is probable that α-crystallin performs a similar role in the eye lens to prevent large-scale fibril formation.

Previous studies have shown that α-crystallin dissociates into its A and B subunits at acidic pH (44, 45), and similar behavior is likely under the conditions employed here to induce amyloid fibril formation. Interaction between αA- and αB-crystallins stabilizes both subunits, particularly the B subunit (6, 9). Thus, dissociation at acidic pH may enable the separate subunits to assemble into fibrils. It has previously been noted that α-crystallin shows a structural transition at 60°C that includes partial unfolding, an increase in high molecular weight aggregate size, and some minor loss of secondary structure (24). This structural transition would further facilitate fibril formation under the conditions used herein. Nonetheless, the observation that a molecular chaperone, which in the case of α-crystallin is renowned for its stability, assembles into amyloid fibrils is consistent with the hypothesis that amyloid formation is a generic property of all of the proteins (37) but one that is mitigated against in the normal lens.

Given the remarkable stability of the protein scaffold within the lens, how could the structure of the crystallins become sufficiently destabilized to induce large-scale formation of amyloid fibrils in disease states? Intuitively, it is possible that the avascular lens environment is conducive to fibril formation for a variety of reasons. 1) The protein concentration is high at ~300 mg/ml. 2) The lens nucleus contains cells and proteins as old as the individual. 3) The lens loses water, and crystallins compact toward its center gradually with time (1). 4) The lens nucleus is acidic with a pH as low as 6.5 (46), all of which may contribute to destabilization of crystallin structures. Moreover, the chaperone ability of α-crystallin is reduced significantly at lower pH values (47, 48), which would favor enhanced protein precipitation including that arising from fibril formation by the other crystallins. There is also less chaperone-active α-crystallin present in the aging lens (4, 19), which would lead to a reduction in its ability to deal with crystallin aggregation, and thereby potentially open up an opportunity for fibril formation indirectly.

The propensity to form amyloid fibrils in the lens is also likely to be increased as a result of factors that cause destabilization of the crystallins. Environmental factors that cause destabilization have previously been linked to cataract formation (1), and under such conditions the crystallin proteins may partially unfold and lose their structural integrity, especially in the lens nucleus. Causative links to cataract formation include genetic mutations that destabilize the crystallins relative to the lens nucleus. Cataract formation has been known to induce the formation of amyloid fibrils in conditions that have not only been implicated in cataract but have also been known to induce the formation of amyloid fibrils in other systems such as the Aβ-peptide (49).
23. McParland, V. J., Kad, N. M., Kalverda, A. P., Brown, A., Kirwin-Jones, P., Hunter, M. G., Sunde, M., and Radford, S. E. (2000) Biochemistry 39, 8735–8746
24. Burgio, M. R., Bennett, P. M., and Koretz, J. F. (2001) Mol. Vis. 7, 228–233
25. Siezen, R. J., Bindels, J. G., and Hoenders, H. J. (1979) Exp. Eye Res. 28, 551–567
26. Augusteyn, R. C., Koretz, J. F., and Schurtenberger, P. (1989) Biochim. Biophys. Acta 999, 293–299
27. Schurtenberger, P., and Augusteyn, R. C. (1991) Biopolymers 31, 1229–1240
28. Augusteyn, R. C., Ellerton, H. D., Putilina, T., and Stevens, A. (1988) Biochim. Biophys. Acta 957, 192–201
29. Poon, S., Rybchyn, M. S., Easterbrook-Smith, S. B., Carver, J. A., Pankhurst, G. J., and Wilson, M. R. (2002) J. Biol. Chem. 277, 39532–39540
30. Snyder, S. W., Ladror, U. W., Wade, W. S., Wang, G. T., Barrett, L. W., Matsuyoshi, R. D., Huffaker, H. I., Krafft, G. A., and Holzman, T. F. (1994) Biophys. J. 67, 1216–1228
31. Lund, A. L., Smith, J. B., and Smith, D. L. (1996) Exp. Eye Res. 62, 651–672
32. Goldstein, L. E., Muffat, J. A., Cherny, R. A., Moir, R. D., Ericsson, M. H., Huang, X., Mavros, C., Coccia, J. A., Faget, K. Y., Fitch, K. A., Masters, C. L., Tanzi, R. E., Chylack, L. T. Jr., and Bush, A. I. (2003) Lancet 361, 1258–1265
33. Pepys, M. B. (2001) Philos. Trans. R. Soc. Lond Biol. Sci. 356, 203–210
34. Srinivas, V., Santoshkumar, P., and Sharma, K. K. (2002) J. Protein Chem. 21, 87–95
35. Fezoui, Y., and Toplow, D. B. (2002) J. Biol. Chem. 277, 36948–36954
36. Kelly, J. W. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 930–932
37. Sunde, M., and Blake, C. (1997) Adv. Protein Chem. 50, 123–150
38. Sunde, M., Serpell, L. C., Bartlam, M., Fraser, P. E., Pepys, M. B., and Blake, C. C. (1997) J. Mol. Biol. 273, 729–739
39. Frederikse, P. H. (2000) Curr. Eye Res. 20, 462–468
40. Kudva, Y. C., Hiddinga, H. J., Butler, P. C., Mueske, C. S., and Eberhardt, N. L. (1997) FEMS Lett. 117, 17–21
41. Stege, G. J., Renkawek, K., Overkamp, P. S., Verschuure, P., van Rijk, A. F., Reijn-Aalbers, A., Boelens, W. C., Bosman, G. J., and de Jong, W. W. (1999) Biochem. Biophys. Res. Commun. 262, 152–156
42. Stevens, A., and Augusteyn, R. C. (1987) Curr. Eye Res. 6, 739–744
43. Poon, S., Rybchyn, M. S., Easterbrook-Smith, S. B., Carver, J. A., Pankhurst, G. J., and Wilson, M. R. (2002) J. Biol. Chem. 277, 39532–39540
44. Snyder, S. W., Ladror, U. W., Wade, W. S., Wang, G. T., Barrett, L. W., Matsuyoshi, R. D., Huffaker, H. I., Krafft, G. A., and Holzman, T. F. (1994) Biophys. J. 67, 1216–1228
45. Lund, A. L., Smith, J. B., and Smith, D. L. (1996) Exp. Eye Res. 63, 661–672
46. David, L. L., Lampi, K. J., Lund, A. L., and Smith, J. B. (1996) J. Biol. Chem. 271, 4275–4279
47. Srivastava, O. P., McEntire, J. E., and Srivastava, K. (1992) Exp. Eye Res. 54, 893–901
48. Goldstein, L. E., Muffat, J. A., Cherny, R. A., Moir, R. D., Ericsson, M. H., Huang, X., Mavros, C., Coccia, J. A., Faget, K. Y., Fitch, K. A., Masters, C. L., Tanzi, R. E., Chylack, L. T. Jr., and Bush, A. I. (2003) Lancet 361, 1258–1265
49. Poon, S., Rybchyn, M. S., Easterbrook-Smith, S. B., Carver, J. A., Pankhurst, G. J., and Wilson, M. R. (2002) J. Biol. Chem. 277, 39532–39540
50. Snyder, S. W., Ladror, U. W., Wade, W. S., Wang, G. T., Barrett, L. W., Matsuyoshi, R. D., Huffaker, H. I., Krafft, G. A., and Holzman, T. F. (1994) Biophys. J. 67, 1216–1228
51. David, L. L., Lampi, K. J., Lund, A. L., and Smith, J. B. (1996) J. Biol. Chem. 271, 4275–4279
52. Srivastava, O. P., McEntire, J. E., and Srivastava, K. (1992) Exp. Eye Res. 54, 893–901
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*J. Biol. Chem.* 2004, 279:3413-3419.
doi: 10.1074/jbc.M308203200 originally published online November 13, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M308203200

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