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Lens Aging: Effects of Crystallins

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Abstract

The primary function of the eye lens is to focus light on the retina. The major proteins in the lens— a, b, and g-crystallins—are constantly subjected to age-related changes such as oxidation, deamidation, truncation, glycation, and methylation. Such age-related modifications are cumulative and affect crystallin structure and function. With time, the modified crystallins aggregate, causing the lens to increasingly scatter light on the retina instead of focusing light on it and causing the lens to lose its transparency gradually and become opaque. Age-related lens opacity, or cataract, is the major cause of blindness worldwide. We review deamidation, and glycation that occur in the lenses during aging keeping in mind the structural and functional changes that these modifications bring about in the proteins. In addition, we review proteolysis and discuss recent observations on how crystallin fragments generated in vivo, through their anti-chaperone activity may cause crystallin aggregation in aging lenses. We also review hyperbaric oxygen treatment induced guinea pig and ‘humanized’ ascorbate transporting mouse models as suitable options for studies on age-related changes in lens proteins.

Keywords

lens crystallins; aging; lens opacity; chaperones; deamidation; glycation; oxidation; peptides

1. Introduction

The composition and architecture of the eye lens, with its elongated fiber cells filled with stable long-lived proteins, allows the lens to focus light on the retina. Although the lens has protective mechanisms to preserve its function throughout the life span of an individual, with aging these mechanisms begin to deteriorate and the lens begins to accumulate modified proteins. This failure leads to gradual aggregation of the lens proteins, with a concomitant loss in the optical quality of the lens, and ultimately leads to opacity, or cataract [1–8]. There are several excellent reviews in the literature that describe both the physiology and biochemistry of the lens and cataract formation [7–18]. These reviews provide well-founded accounts of the mechanisms of lens development, lens morphogenesis, lens protein oxidation, presbyopia, and the biology of lens capsule. The purpose of this review is to emphasize the recent findings on age-related changes in lens crystallins and how such changes might affect the structure and function of lens crystallins and modulate aggregation of crystallins, a hallmark of aged and cataract lenses.

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2. Lens development, anatomy and physiology

2.1. The embryonic lens

The eye lens is an a vascular tissue encapsulated in a collagenous basement membrane-like material composed of a single layer of epithelial cells on the anterior subcapsular surface [19]. The lens derives all of its nutrients and oxygen from the aqueous humor and vitreous body. At the equatorial zone the epithelial cells begin to differentiate, elongating to become fiber cells, during which time they lose the organelles, and begin synthesizing large quantities of the structural proteins called crystallins. This process continues throughout life, though at a slower pace, with the younger/newer fiber cells pushing the older fibers to the center (nuclear) region of the lens (Fig. 1). Although the lens has a limited number of cells and cell types, its development is complex [5,12]. Lens development begins with the invagination of lens placode toward the optic cup to become the lens pit. The invagination process continues, the pit closes and a lens vesicle is formed in humans by embryonic day 33. Following this, the differentiating epithelial cells start filling the vesicle until the whole cavity is obliterated with fiber cells by the end of seventh week. The 'first formed' fiber cells that occupy the center of the lens become the embryonic nucleus [12,19]. Lens development is not complete without the removal of all potential light-scattering organelles from the fiber cells, which is accomplished in a programmed process that involves proteases [15,20]. The embryonic spherical lens, measuring about 0.35 mm in diameter initially, quickly begins to grow to an elliptical organ of about 35 mg by birth. Studies of human lenses ranging in age from 6 months to 99 years show that the lens actually grows in two phases, an "asymptotic phase" during the prenatal and early childhood periods and a linear phase during the rest of the life span [5,21]. Although it was once thought that lenses of males are heavier than lenses of females, new data do not support this notion [21]. The best estimate of human lens weight can be obtained by the expression, $W=1.38A_b+149\exp^{[\exp^{(1.6-3A_c)}]}$, where W is lens weight in mg, A_b is post-natal age in years and A_c is the time since conception in years [21]. Because the lens is composed of a range of fibers representing different ages, it is an attractive tissue for studying the effects of aging on protein structure and function [4].

2.2 The lens capsule and epithelium

The lens capsule is a specialized basement membrane with lamellar structure formed anteriorly by epithelial cells and posteriorly by fiber cells. Collagen type IV, laminin, entactin, heparin sulfate proteoglycan, and fibronectin constitute the major components of the capsule and contribute to the molding of lens shape [5]. The lens capsule, which is about 4 mm in thickness at the birth, grows throughout life but as age advances, its thickness reaches as high as 30 mm at the peripheral region of the anterior capsule [8]. The mechanical properties of the lens capsule decrease with age, which can be attributed to the changes occurring in the capsule collagen. All aspects of the lens capsule have been described in an excellent, recently published review [17]. A single layer of polygonal cuboidal epithelial cells cover the lens anteriorly and can be demarcated into two regions, namely, the non-dividing central epithelium and dividing [germinative] epithelium, which in turn differentiate at the bow region (Fig. 1). The lens epithelial cells possess the remarkable feature of dividing and differentiating into new fiber cells throughout life but this process diminishes with age. The lens epithelium is the major site of transport, metabolism and detoxification, since the lens fiber cells derive the bulk of their nutrients through the epithelial cells. An age-related decrease in epithelial density has been documented [22]. However, it has not been established that low epithelial cell density contributes to the development of cataract [23]. The epithelial cells are known to provide the lens with the first line of defense against oxidative insults [8,14]. Because the integrity of the epithelial layer is critical to normal lens physiology, epithelial cell death by apoptosis or any abnormalities in the cytoskeleton proteins in these cells are detrimental to the underlying fiber cells. Results of the studies of the effects of different metabolites and drugs on the epithelial

cultures (primary or transformed) have been inconclusive in elucidating the exact role of the epithelial cells in inducing age-related changes of the lens because the cell culture system does not replicate the gradient of cellular and metabolic differences observed in lens [7,8,24]. A recent review [14] on lens epithelia has summarized the role of aA- and aB- in lens epithelia and has highlighted that the a-crystallin expression is critical for the survival, growth and proliferation of this group of cells.

3. Lens Proteins and Their Role in Lens Aging

3.1. Lens water-soluble and water-insoluble proteins

The human lens is composed of three main types of proteins, a-, b- and g-crystallins, which account for nearly 90 percent of the total lens proteins [4,11]. Homogenization of human lens in water or buffer and centrifugation yields two fractions: a water-soluble (WS) fraction and a water-insoluble (WIS) fraction [4,5]. As much as 50 percent of the lens proteins in the aged (>50 yrs) human eye enters the WIS fraction [3,25], and WIS proteins are known to increase with aging [4,5]. Although it has been known for over 30 years that almost all of the a-crystallin present in the nuclear region of +40-year-old lenses exists in the WIS fraction, [3], the reasons for the insolubilization of a-crystallin in the nuclear region are only now becoming clear. Compared with the WS fraction, the WIS fraction contains a relatively larger proportion of the modified and cross-linked crystallins. In addition, the WIS fraction also contains lens fiber cell membranes and cytoskeleton proteins [5,26]. The crystallins in the WIS fraction are the primary light-scattering aggregates in aging lenses. Studies show that in human lens, the nuclear region, the oldest part of the lens, has the highest percentage of WIS proteins and the proteins from the nuclear region also exhibit the highest degree of modifications and light-scattering properties [5,6,26–35]. Analyses of the WS protein fraction by size exclusion chromatography and dynamic light-scattering methods show that aged lenses have a significant reduction in the WS protein fraction as compared to young lenses (Fig. 2). The crystallins in the human lens WS fraction can be categorized into three major peaks: a-, b- and g-crystallins (Fig. 2). The mass distribution indicates that the aged lens contains WS protein aggregates as large as 3500 kDa, as compared to 2000 kDa aggregates in the young lens [36]. The bulk of the WS protein in aged lenses (that is, 70 years old) is comprised of b- and g-crystallins, whereas in a 19-year-old lens, a-, b- and g-crystallin are present in the WS fraction in comparable concentrations [36].

3.2. a-Crystallin and its A and B subunits

Of the three classes of crystallins, a-crystallin is the predominant type (Table 1) and is composed of two types of subunits, A and B, which noncovalently associate to form aggregates with an average molecular weight of 800 kDa [11]. The aA-crystallin subunit is more prevalent than the aB- subunit, in a ratio varying from 3:1 to 2:1 in different regions of the lens. The primary structures of aA- and aB-crystallin subunits exhibit a high degree of homology between them and to small heat shock proteins [sHSPs] because of the conserved a-crystallin domain in these proteins [9,11]. The secondary structure of a-crystallin subunits is primarily in the form of b- sheets [9]. The tertiary and quaternary structures of a-crystallin are not known. The aA-crystallin domain is thought to have an immunoglobulin-like folding pattern [11]. Nuclear magnetic resonance studies show that the C-terminal regions of aA- and aB-crystallins are more flexible than the N-terminal regions [37]. The a-crystallin molecule is a dynamic oligomer, with the subunits dissociating and reassociating constantly. For example, in four hours time, all subunits in a a-crystallin molecule exchange with a new set of subunits [38]. This subunit exchange property is also implicated in the α -crystallin chaperone activity. Several studies have shown that the subunit exchange rate is affected by specific mutations in a-crystallin subunits [39,40] or is altered after truncations [40,41] and age-related modifications [40,42,43]. Both in vivo and in vitro studies to understand the molecular basis of cataract-

causing crystallin mutations and age-related cataract show that loss of chaperone activity and increased protein aggregation are responsible for the lens pathology [44–47]. Delineation of the three-dimensional structure of the major constituent of lens proteins, a -crystallin, has thus far eluded scientific investigation. However, the availability of structures of sHSP 16.1 [48] and 16.5 proteins [49], which have a high sequence homology to aB- and aA- sequences and structures allows researchers to build structural models on which to base valid structure-function predictions [11,50]. The use of site-directed mutagenesis, Cryo-EM and NMR techniques in combination should allow the researchers to build high-resolution structures for aA- and aB-crystallin oligomers.

3.3. β -crystallin and g -crystallin

Both b- and g -crystallins are comprised of several proteins related by sequence and structure [11]. The b-crystallins include four acidic and three basic subunits that have molecular masses in the range of 22 – 28 kDa, whereas γ -crystallins have 7 different types of subunits of ~20kDa [Table 1]. The gS-crystallin elutes ahead of the rest of g-crystallins during size exclusion chromatography although all g-crystallins are considered to be monomeric in nature. In human lenses, the gD- and gE-crystallins are not expressed, whereas they are found in bovine lenses. The primary structures of all 3 classes of crystallins have been known for some time, as have the three-dimensional structures of β - and g-crystallins. A recent review provides a good discussion of the structures of b- and g -crystallins [11].

A primary function of crystallins is to focus light on the retina by maintaining the necessary refractive characteristics and clarity of the lens. Lens proteins undergo very little turnover, but they do undergo various changes during aging and cataractogenesis, including increased crystallin proteolysis, fragmentation and aggregation. Binding of denatured b- or g-crystallins to either of the a -crystallin subunits affects the subunit exchange [51]. Proper subunit interaction between crystallins is necessary to prevent the formation of light-scattering aggregates of crystallins. Age-related changes may affect the short-range interactions [52] among a-, b- and g -crystallins that are critical for lens transparency. Equilibrium dialysis studies show that an age-related decrease occurs in the interactions between g-crystallin and a -crystallin [53]. The changes in the interactions may lead to increased homoaggregation of proteins and localized fluctuations in protein densities sufficient to cause light scattering [53, 54]. The formation of protein aggregates of crystallins, either by this mechanism or other mechanisms, could be the first event in the development of cataracts.

4. Role of Crystallins in Lens Transparency and Lens Opacity

4.1. α -Crystallin chaperone activity

There is general agreement that in addition to the structural role of a -crystallin in the lens, it also functions as a chaperone [11,44,55,56]. Studies of total lens homogenates as well as of whole lens also argue in favor of the chaperone function of a -crystallin in the lens [55,57]. The aA-knockout studies as well as analysis of congenital cataracts with aA- and aB-crystallin mutations also support the role of these two subunits in a -crystallin chaperone activity [46, 47,58–60]. a -Crystallin chaperone activity (or that of its subunits) can suppress the aggregation of proteins denatured by oxidation, heat, and other stressors [55]. Chaperone activity is susceptible to modulation by low-molecular-weight compounds such as adenosine triphosphate (ATP) and glutathione [61,62]. Concentrations of ATP and glutathione are known to change with aging [10,63]. Glycation, ultraviolet irradiation and deamidation results in loss of a -crystallin chaperone activity [40,42,43,64]. Glycation from uncontrolled diabetes and exposure to ultraviolet light are known to increase the cataract risk [64,65]. The factors that suppress a -crystallin chaperone function are thought to accelerate aggregation of other crystallins that are undergoing age-related modifications and losing their native structure.

Consistent with this hypothesis, decreased chaperone activity, increased crystallin aggregation, light scattering and loss of lens transparency have indeed been demonstrated in aged human lenses [66].

Both direct and indirect evidence suggests that multiple regions in a -crystallin subunits may be involved in chaperone activity [67–69]. The structural changes in the modified crystallins are likely to 1) mask the chaperone site and prevent chaperone action, 2) interfere with aA- and aB-crystallin subunit interactions, or 3) make the chaperone protein “hyperactive,” resulting in a chaperone-substrate complex that is unstable and aggregates rapidly [45,70]. The structure of a -crystallin apparently must remain dynamic, with subunits constantly disassociating and reassociating, in order for chaperone activity to be maintained. For example, crystallin cross-linking studies show that when subunit dissociation is restricted, chaperone-like activity decreases [66,71]. Conversely, cleavage of a -crystallin intersubunit cross-links results in recovery of chaperone activity [66], indicating that the dynamic nature of the a-crystallin structure needs to be maintained in order to exert maximal chaperone activity. Therefore, increased crosslinking of a -crystallin, which is known to occur in the aging lens [4], may be a contributing factor in the loss of chaperone activity as the lens ages.

4.2. Post-translational modification of crystallins

Studies have shown that lens crystallins undergo extensive modifications [4,33–35,72–76]. Recent advances in analytical methods have allowed the identification of several new modification sites. The major modifications include deamidation, truncation, Cys-methylation, phosphorylation, oxidation, acetylation, carbamylation, and glycation [26,34,35,64,77–84]. Various modifications and modification sites are summarized in the Table 2. While a number of studies have aimed at identifying the modifications and the sites of these modifications, few studies have attempted to measure the extent of the modifications. Most studies have compared the level of modifications in young and aged lenses or in young and aged, cataract lenses. In some instances, the modifications were identified in pooled lens samples, but the values in pooled lens samples do not reflect the sample-to-sample variations generally observed in individual human lens samples. For these reasons one can see inconsistency in some of the modifications reported in the literature. This is obvious from the references in Table 2 which shows that deamidation or oxidation or phosphorylation at specific sites were not discovered by each and every analysis.

4.3. Deamidation of crystallin affects both structure and function

While several studies have reported deamidation of a-, b- and g-crystallins isolated from young, aged and cataract lenses, the results are not always consistent with respect to deamidation sites in these proteins [33–35]. Crystallins isolated from young, noncataract lenses also contain significant deamidations at specific sites, and studies have shown that the amount of deamidation at these sites increases with age [34,35,85–90]. In addition, crystallins isolated from aged and cataract lenses show deamination at additional sites and in higher levels [34, 87,89]. However, the specific role of deamidation in lens protein aggregation and cataractogenesis is not fully understood because the deamidated proteins also often have other modifications such as truncation, glycation, and oxidation [33–35]. In one study deamidation of lens proteins was more frequent at glutamine sites than at asparagine sites, [34] whereas the reverse was true in another study [35]. Different levels of transglutaminase activity in the lenses used in these studies may in part, explain the discrepancy, since it has been reported that transglutaminase present in lens can also deaminate glutamine [91].

Several studies were carried out recently to determine the role of deamidation at the molecular level [40,42,43,92–94]. Investigators have taken advantage of the chaperone-like activity of aA- and aB-crystallins to determine the effect of modifications on the function of a-crystallin

subunits. Deamidation introduces a negative charge to a protein, and protein deamidation causes changes in protein tertiary structure and, in turn, affects structural and functional properties [42,94]. Deamidation of specific residues in human lenses is higher in the water-insoluble [WI] proteins than in the water-soluble [WS] proteins [34]. This observation suggests that deamidation might be leading to structural instability, which in turn causes insolubilization of a protein. The cataract-specific deamidation of N-143 in γ S-crystallin further supports this possibility [95]. A study of age-related deamidation of α A- and α B-crystallins examined the relative effects of deamidation α A-N101D, α A-N123D, or α A-N101/123D on the structural and functional properties of these crystallins [40,42]. Far-ultraviolet (UV) circular dichroism spectral analyses generally showed an increase in the β -sheet contents in α A mutants with deamidation. Intrinsic tryptophan (Trp) and total fluorescence spectral studies suggested altered microenvironments in the deamidated α A- mutants. ANS [8-anilino-1-naphthalenesulfate] binding study showed generally increased fluorescence, with a blue shift on deletion of the N-terminal domain in the deamidated mutant proteins. Overall, it was found that, relative to wild-type (WT)- α A- homomers, the mutant proteins exhibited major structural and functional changes. The maximum decrease in chaperone activity in homomers occurred upon deamidation at the N123 residue. These authors showed that deamidation alone exerted a greater effect on chaperone activity than the deletion of the N-terminal domain or C-terminal extension of α A-crystallin. There is evidence for *in vivo* truncation of an A-crystallin at both C- and N-terminal regions [9,33,96]. Interestingly, a previous study also showed that the loss of chaperone activity in the N123 deamidated A-crystallin was substantially restored when the protein was truncated at either the C- or N-terminal. In the absence of deamidation, N- and C-terminal truncation was found to lead to decreased chaperone activity [40]. The chaperone activity of the deamidated α A- and α B-crystallins at other sites and with combination of truncation at either C- or N-terminal gave mixed results. Therefore the net contributions of deamination and truncations to decreased chaperone activity in aging lenses is not known because α A-crystallin is deamidated at a number of sites and a significant portion of the protein also exists in truncated form [9,34]. In addition, *in vitro* studies show that deamidation also affects the structure-function of a B-crystallin [43]. Deamidation of N146 but not of N78 in human α B-crystallin has profound effects on the structural and functional properties of the protein [43]. The heteroaggregates of wild-type α A- or α B-crystallins and deamidated forms of these two crystallins have decreased hydrophobicity, increased oligomer size, altered structure and decreased chaperone activity [42,43]. These results, obtained with recombinant proteins, are in contrast to an earlier report in which increased hydrophobicity was observed in high molecular weight crystallin aggregates from lenses that generally have deamidated α A- and α B-crystallins [34,35,56]. It should also be pointed out that while earlier studies appeared to establish a correlation between hydrophobicity and chaperone activity of a β -crystallin [97, 98], subsequent studies show that this is not always the case with several mutants of α A- and α B-crystallins [99,100]. An explanation for this discrepancy is yet to find its way into literature.

The lack of easily measurable biological activity in b- and g-crystallins has hindered the precise measurement of the effects of age-dependent modifications on these groups of crystallins. To circumvent this limitation, attempts have been made to correlate the modifications to the structure and stability of b- and g-crystallins. Since deamidation is thought to be a major age-related modification in b-crystallins, deamidated mimics of bB1- [92], bB2- [94] and bA3-crystallins [101] have been studied in an effort to understand age-related changes in β -crystallins. These crystallins undergo *in vivo* deamidation and the extent of deamidation is higher in aged and cataract lenses [34]. Human bB1 Gln204Glu was found to be less stable than the wild-type protein [92]. In a related study, deamidation of bB1Gln146 was also found to be a destabilizing reaction [102]. The deamidation of bB2, the most abundant human β -crystallin, was studied at Q162 and Q70 by creating a double mutant Q70E/Q162E [94]. The recombinant protein failed to refold after urea induced-unfolding, suggesting that alteration in the charges at the monomer-monomer interface destabilizes the protein. In support of this view,

the authors also found changes in the tertiary structure of the protein following deamidation. Deamidation of α A3-crystallin at Q180 in the C-terminal domain and at Q85 in the N-terminal domain was found to lead to destabilization and aggregation of the protein. Deamidated α A3-crystallin showed significant changes in the tertiary structure, whereas the difference between the secondary structure of the wild-type proteins and that of mutant proteins was minimal. In another study the role of several surface Gln and Asn residues in the stability of α A3-crystallin was investigated. Deamidation at the C-terminal domain (N133 and N155) was found to have greater effect on the protein structure than deamidation at N-terminal residues (Q42 and N54) [101]. Furthermore, deamidation also resulted in aggregation of α A3-crystallins and in interaction between β A3- and β B1-crystallin subunits. The data support the hypothesis that in vivo deamidation may affect the stability of the protein and that age-related accumulation of deamidated β -crystallins may contribute to crystallin insolubilization in vivo.

All deamidation effect studies were carried out using 'mimics' at 100 percent concentration. However, in vivo, the crystallins are only partially deamidated and the same crystallins have other modifications. From the studies on α A-crystallin, which has truncation and deamidation, we know that multiple post-translational modifications may lead to unpredictable effect [40]. Therefore, from 'in vitro' studies it is not clear whether the true impact of in vivo deamidation at a specific site can be determined. A transgenic approach involving expression of deamidated forms of crystallins may offer an opportunity to test the in vivo effect of deamidation at specific sites.

4.4. Advanced glycation endproducts (AGE) affect crystallin structure-function

Modification of crystallins by dicarbonyls and sugar derivatives is well documented [64,80, 83,103–111]. Evidence is increasing that ascorbate oxidation products have a role in in vivo glycation [80,110,111]. Table 3 lists glycation modifications and cross-links isolated from aged and cataract human lenses. The readers should refer to the citations for the levels of AGE's in lens under different conditions. In general, the concentrations of most of the AGE's increases with age. While some glycation reactions involve direct or simple modification of arginine (Arg), lysine (Lys) and cysteine (Cys) residues, other glycation reactions generate crosslinks that affect the dynamic state of α -crystallin. Estimates are that <1% of the total Lys and Arg in crystallins are modified in vivo [112]. The vast majority of the modifications involving Lys and Arg are brought about by sugars or their oxidation products [80,83,105,113–116]. While a strong correlation between advanced glycation endproducts (AGE) formation in lens crystallins and overall deterioration of lens transparency was established years ago, the precise changes that occur in lens crystallin structure after modifications have only recently been elucidated [56,117–119]. A recent study showed that modification of α B-crystallin at a single site is sufficient to alter its structure-function [118]. In this study the authors used a cysteine mutant of α B-crystallin (α BK90C) and site-specific modification of the Cys to get an analog of the AGE OP-lysine [2-Ammonio-6-[3-oxidopyridinium-1-yl]hexanoate] at K90 site of the protein (Fig. 3). Lys 90 is near the chaperone site of α B-crystallin [120]. OP-lysine modification is one of the most abundant AGE modifications in both aged and cataractous lenses [121]. As a control, α BK90C was subjected to aminoethylation to obtain α BK90C-AE. The control protein used in this study retained the positive charge in the mutant protein that ordinarily is lost during the mutation. The authors found that the α BK90C-OP-lysine has diminished chaperone activity (Fig. 4), decreased intrinsic tryptophan fluorescence and bis-ANS binding. The modified protein, however, exhibited no significant alteration in secondary, tertiary and quaternary structures. On the other hand, the control protein α BK90C-AE behaved like wild-type α B-crystallin with respect chaperone activity and structure. The α BK90C-OP study showed, for the first time, that the introduction of a single AGE near the chaperone site diminishes anti-aggregation properties of α B-crystallin, even when the changes in the protein structure are minor. In other studies, extensive modification of α A- and α B-crystallins with

glycating agents was shown to lead to aggregation of the crystallins and decreased chaperone activity [56,117,119,122]. The mapping of glycation sites in aA- and aB-crystallin has shown differential susceptibility of Lys and Arg residues [123]. The susceptibility of the residues to glycation and the influence of neighboring residues are attributed to the different glycation rate. Glycation reactions culminating in crosslinks between a-crystallin subunits may also be affecting the chaperone activity by affecting the dynamic state of the protein required for chaperone activity [66,71]. Since under physiological conditions glycation reactions continue to occur in vivo, it can be argued that with aging, the modifications begin to accumulate and reduce the chaperone function of a-crystallin.

While most glycation studies show a decrease in the chaperone activity of a-crystallin following glycation, [117,119] one study revealed that modification of a-crystallin with a low concentration of methylglyoxal enhances chaperone activity, to the tune of 40 – 60 percent [124,125]. The chaperone sites involved in the binding of increased amounts of client (substrate) proteins are yet to be delineated. Immunochemical studies have established that methylglyoxal modification does indeed occur in normal lenses and that these modifications accumulate with aging of lens proteins [83,109,113]. Limited modification of a-crystallin with methylglyoxal perturbs the protein structure and increases the surface hydrophobicity [124]. Increased surface hydrophobicity has been shown to correlate with increased chaperone activity of a-crystallin [97]. Methylglyoxal reacts with Arg, Cys and Lys to produce stable adducts and cross-links and is one of the glycating dicarbonyls in the lens [83,109,113,125]. It has been shown that Cys residues in aA-crystallin are not involved in methylglyoxal-mediated enhancement of chaperone activity [126]. The neutralization of the positive charge on Arg by methylglyoxal is apparently responsible for the enhanced chaperone activity of a-crystallin [127]. Additionally, the methylglyoxal reaction was also found to block glycation of a-crystallin by ascorbate and ribose. The increased chaperone activity after modification with methylglyoxal prompted the investigators to propose that “low levels of methylglyoxal might help the lens remain transparent during aging” [125]. It is yet to be determined whether the methylglyoxal effect can be sustained if crystallins are also modified by other mechanisms, such as deamidation, phosphorylation or truncations, which are continuously occurring in vivo [34]. Ascorbate oxidation product erythrose is a powerful glycating agent and there is some evidence for its involvement in lens crystallin glycation [110,114,115]. The glycation-induced aggregation of crystallins similar to the aggregation seen in aged lens has been shown by several studies [80,116,128].

4.5. Oxidation, phosphorylation and other modifications affect crystallin structure-function

Oxidation is considered to be a major physiological challenge to lens proteins, and recent reviews have discussed the role of oxidation in cataract [16,18,129,130]. The lens appears to be subjected to increasing oxidative stress with aging because of the progressively weakening antioxidant system. The decline in the antioxidant activities of the lens is manifested by decreased glutathione levels, reduced efficiency of enzymes involved in restoring reduced glutathione levels, the accumulation of proteins with disulphides and mixed disulphides, and oxidation of Met residues [4,5,8,33–35]. While aA-crystallin has only two Cys residues susceptible to oxidation, b- and g-crystallins have several Cys residues that are susceptible to oxidation (Table 2). Although Trp and His are susceptible to oxidation, strong evidence is lacking for their oxidation in aging lenses, yet these amino acids are oxidized in advanced cataracts [129]. Free radicals – hydroxyl radical, superoxide and singlet oxygen— are the likely oxidizing species in vivo [131–134]. To study the role of α -crystallin oxidation in lens aging, investigators have generated protein aggregates in vitro, similar to those found in aged lenses, by oxidation of SH- groups in aA-, b- and g-crystallins. Oxidation of aA- and aB-crystallin has been shown to lead to structural changes and loss of chaperone activity [135]. Methionine oxidation in aging lens has been linked to cataractogenesis [136]. Recently loss of chaperone

activity in α A-crystallin due to oxidation of Met was demonstrated and this loss was reversed by methionine sulfoxide reductase [137]. Lens crystallins have several phosphorylation sites (Table 2), and some studies have shown that phosphorylation can modulate chaperone activity of α -crystallins [138–140]. There is no evidence of age-related changes in the extent of phosphorylation of crystallins, but one study demonstrated that α B-crystallin from cataract lens was not phosphorylated [84]. The significance of this absence of phosphorylated α B-crystallin is yet to be determined. Methylation of Cys residues in crystallins has been reported in several studies [34,141–144] but age-related changes in this reaction have not been adequately elucidated. It has been hypothesized that Cys methylation may be a protective mechanism to prevent disulphide formation and aggregation of crystallins [145]. *In vivo* modification of human lens crystallins by 3-hydroxykynurenine glucoside, kynurenine and 3-hydroxykynurenine has been reported [112,146–148]. Since kynurenine reacted with His83 at the chaperone site of α B-crystallin it was proposed that this reaction might affect the chaperone activity in aging lenses [146]. However, the structural consequence of this or other modifications with kynurenine is yet to be determined. In addition, modification of crystallins by *n*-formylkynurenine has been documented in lens [149] and this modification may also result in both structural and functional alterations of crystallins.

4.6. Proteolysis in lens—from morphogenesis to aging

Lens proteases play a critical role during lens development and morphogenesis. Several proteases have been isolated from lenses [150–161]. In addition to these proteases, the lens also contains a whole set of proteases involved in organelle degradation [15,20,162–164]. The ubiquitin-dependent proteolytic pathway studied in lens is involved in degradation of oxidatively modified proteins [165,166]. However, due to age-related decrease in ubiquitin conjugation activity the oxidatively modified crystallins accumulate in lens, primarily in the nuclear region. This in turn is believed to be responsible, at least, in part to age-related protein aggregation and light scattering [166]. Recent studies have shown that inhibition of the proteasome activity involved in differentiation of lens fiber cells prevents normal maturation of fiber cells [164]. Calpains have been implicated in the maturation of rodent lens crystallins [167] and in the development of cataract in sheep [168] but their role in human cataractogenesis is yet to be established, primarily because of the presence of a calpain inhibitor in the lens that is at a several fold higher concentration than calpain itself [154,169,170]. The presence of lens proteins cleaved at specific sites during aging [9,171] provides credence to the statement that, although protease activity begins to decline in lens fibers as they mature, the lens has appreciable levels of protease and peptidase activity [158,172]. The major bonds cleaved *in vivo* are on the carboxyl side of Asn101, Asp151, Ser168 and Ser169 of the A chain and on the carboxyl side of Thr170 of the B chain of bovine α -crystallin [9,171]. The other truncation observed in α A- and α B-crystallin is due to cleavage at the carboxyl side of Ser172, [33] and the amount of cleaved α A- and α B-crystallin is high in the WIS fraction. One study found that in aged human lenses, α A-crystallin is truncated by 3 residues and α B is truncated by 1 or 6 residues at the N-terminus [96]. Mass spectrometric analysis of WS and WIS fractions has identified several of the previously known truncations as well as a few additional ones [26,34,96,173]. But the protease[s] responsible for α -crystallin truncation at multiple sites has not been identified. Truncation of bB1-, bA3/A1- and bB2-crystallins *in vivo* has been reported based on the identification of several truncated and cleaved b- and g-crystallins in both WS and WIS fractions of human lenses [26,173]. Since truncation affects the stability of bB2-crystallin *in vitro*, [94] it is hypothesized that *in vivo* truncation of the protein may be responsible for its aggregation with aging.

4.7. Truncation effect on oligomerization of crystallin subunits and the chaperone activity of crystallin

The lens WIS fraction of crystallin, which contains mostly a-crystallin aggregates (with some b- and g-crystallins, [3,26,30] is known to have increased amounts of C-terminally truncated aA- and aB-crystallins as compared to the WS fraction [30,31,174]. Recent studies have shown that C-terminal truncation affects oligomerization and subunit exchange, [41] as demonstrated by recombinant human aA¹⁵¹ and wild-type aA-crystallins. The aA¹⁵¹ form is found among the several truncated forms of crystallins in aged lenses [11,40]. In our study of the effects of truncation on oligomerization of crystallin subunits, we mixed aA¹⁵¹ with wild-type proteins in different proportions and found a concentration-dependent increase in the oligomeric mass for the hetero-oligomer (Table 4). At an aA: aA¹⁵¹ ratio of 3:1, the mass increased 2-fold compared to the mass for wild-type protein mixtures. Incubation of aA and aA¹⁵¹ in 2:1 ratio led to hetero-oligomers that were ~ 3 times larger than oligomers formed from aA-wild-type protein. Since aA¹⁵¹ aggregated by itself, we could not reliably measure the size of the oligomer. These results, along with others, [46,175–177] suggest that truncated form[s] of aA- and aB-crystallin may hold the key to the formation of larger aggregates *in vivo*. Fluorescence resonance energy transfer [FRET] experiments involving labeled aA- and aB- crystallins and truncated forms of these proteins showed that truncation affects aA- and aB-crystallin subunit exchange [40,41]. The demonstration of the abnormal behavior of truncated crystallins is highly significant since it provides insight into mechanisms by which cataract is formed in the aB-mutation involving the genesis of a stop codon at 151 position of aB-crystallin [46]. C-terminal truncation of aA- and aB-crystallin with removal of 1–16 amino acids also affects the chaperone activity of these two subunits [175,176]. It is likely that the truncation partially removes the sequence in these subunits that is responsible for keeping the chaperone-substrate complexes in soluble form.

4.8. Low molecular weight crystallin fragments in aging human lens

We have previously shown that the amount of crystallin fragments in older lens fibers, as well as in the WIS fraction, is greater than the amount in younger lens fiber cells and in the WS fraction [152,157]. The amount of degraded crystallin [2.8 to 18 kDa] can be as much as 20% in aged/cataract lenses [78,178]. Crystallin truncation and fragmentation is also seen in bovine lens tissue imaging studies [179,180]. While other investigators have focused on truncated crystallins that have lost few amino acids [74,175,176,181] or that are in 2.8 to 18 kDa range [78,174,178], we have been investigating the low-molecular-weight crystallin fragments and have isolated and identified in human lenses over 25 different crystallin fragments of less than 3.5 kDa. (Table 2 in ref [36]). Among the crystallin fragments we have identified, several are derived from aA-, aB- and bB3-crystallins, including residues from aA- and aB-crystallin subunit interaction regions [68,182,183] or chaperone sites [67,68,120]. We found that the total amount of crystallin fragments is 3- to 4-fold higher in aged lenses. Further, the concentration of peptides is higher in the nuclear region of the lens which represents the aged portion of the lens. The nuclear region of the lens, which has the highest WIS protein content [5], also was found to have the greatest amount of peptides or shorter crystallins [152,157]. This was also illustrated by protein-image analysis studies. When whole-lens cross sections were subjected to MALDI imaging [179,180] to determine the relative distribution of specific crystallin fragments in a given lens, once again the low molecular weight crystallin fragments were found in increasing quantities in the nuclear region (Fig. 5 for representative distribution of 3–5 kDa peptides). At present, it is not known how these peptides are formed, nor is it known why they are not completely hydrolyzed *in vivo* since the lens has appreciable levels of aminopeptidase activity [152,172]. These observations raise important questions: What is the role of crystallin fragments in high molecular weight aggregate formation? What are the effects of crystallin-derived peptides on chaperone activity of a-crystallin? What is the effect of peptides on a-

crystallin subunit interaction since subunit interaction has a role in the chaperone activity of α -crystallin?

4.9. Anti-chaperone activity of crystallin fragments and α -crystallin aggregation

The age-related accumulations of crystallin breakdown products are believed to have a role in the loss of lens transparency and the eventual development of lens opacity [184]. Earlier we proposed that crystallin fragments generated from the oxidized crystallins in aging lenses may enhance the aggregation of denaturing proteins and interfere with the chaperone activity of α -crystallin [185]. In support of this hypothesis, it was shown that trypsin digestion of in vitro oxidized β -crystallin generates a peptide that has anti-chaperone activity [185]. The oxidized peptide binding enhanced the aggregation of denaturing β L- and γ -crystallins [186,187]. The potential role of in vivo-generated crystallin fragments in masking the chaperone activity was investigated using both synthetic α -crystallin fragments and crystallin fragments isolated from aged human lenses [36]. The studies showed that denaturing β - and γ -crystallins aggregate in the presence of peptides from human lenses. A detailed analysis of the interaction of 3 representative peptides — α B¹⁻¹⁸ (MDIAIHHPWIRRPFFPFH), β A3/A1¹⁰²⁻¹¹⁷ (SNAYHIERLMSFRPIC) and γ S¹⁶⁷⁻¹⁷⁸ (SPAVQSFRRIVE)— with denaturing proteins showed their ability to enhance protein aggregation and inhibit chaperone activity of α -crystallin (Fig. 6). α B¹⁻¹⁸ and β A3/A1¹⁰²⁻¹¹⁷ were found to enhance the aggregation of denaturing proteins, whereas γ S¹⁶⁷⁻¹⁷⁸ did not affect protein aggregation. Additionally, both α B and β A3/A1 peptides showed chaperone inhibition activity [36,188]. Crosslinking studies have confirmed the interaction of β A3/A1¹⁰²⁻¹¹⁷ at the chaperone site of α B-crystallin [188]. This study is the first to show that a sequence within β A3/A1-crystallin may become an anti-chaperone peptide (a peptide interfering in chaperone activity) when it is removed from its parent protein. This study is the first report of in vivo accumulating peptides in aging lenses that may be, in part, responsible for in vivo protein aggregation and loss of chaperone activity. Additionally, other peptides present in lenses, originating from the subunit interaction regions, may affect native protein subunit interactions and short-range order interactions [52,54] required to maintain lens transparency.

5. Pathway of Crystallin Fragmentation and Aggregation in Lens Aging

5.1 Pathways for age-related crystallin aggregation and the development of opacity

The age-related increase in crystallin aggregation and loss of lens transparency is well documented [6,8,11]. Aging of lens crystallins is accompanied by proteolysis and several other post-translational modifications in both native protein and truncated crystallins. As shown in the Fig. 7, these changes are proposed to be the driving force that induces conformational change in crystallins. At the same time, crystallin modifications and truncations affect the chaperone function of α -crystallin. These events lead to further aggregation of β - and γ -crystallins and masking of the chaperone sites in α -crystallin by the anti-chaperone peptides. In this scheme crystallin fragments accumulating in the lens during aging occupy the central position in the pathway for lens opacification due to their ability to induce aggregation and precipitation of α A- and α B-crystallin and their anti-chaperone activity [36,188]. Chaperone activity of α A- and α B-crystallin is believed to be responsible for maintaining lens transparency [55,57]. In animal models disruption of chaperone activity has been shown to result in lens protein aggregation and opacity. While the scheme in Fig. 7 does not include various risk factors for post-translational modification of crystallins, [16,64,65,83,129,136] it is assumed that during lifetime an individual is exposed to most of the risks, albeit at low level.

5.2. Animal models for studies of lens aging

Studies of the physiology and biochemistry of the lens show that the age-related changes taking place in the lens occur in an orderly way and at a slow pace over the lifetime of an individual,

whether it is a human or an animal. The *in vivo* changes accumulate, and the aged lens constitutes a collection of these changes. Therefore, it is very difficult, if not impossible, to replicate these changes in a shorter time frame under laboratory conditions using what is considered to be physiological concentrations of reagents. Immortalized lens epithelial cells, primary culture cells and lens organ cultures have been used to study the age-related lens changes. The results of such studies, however, often come under criticism because 1) the studies require the use of 10- to 100-fold more reagents, 2) there is a significant difference in the protein profile of immortalized and primary lens epithelial cells [24] and 3) the epithelial cells do not satisfy the functional obligations as the cells in contact with fiber cells.

Guinea pig lens is similar in most aspects to human lens and may serve as a good model for investigating the mechanisms of lens opacification and cataract formation. Long-term hyperbaric oxygen treatment is used to induce nuclear lens opacity in the guinea pig in an attempt to replicate the development of lens opacity in the human lens [189,190]. Analysis of hyperbaric oxygen-treated lenses shows glutathione loss, increased protein degradation, protein aggregation and insolubilization, protein cross linking, membrane abnormalities, etc, features like the changes observed in the aging human lens [189–192]. For these reasons it appears that this model is suitable to study the molecular mechanisms of age-related lens opacification and cataract. It remains to be seen whether crystallin fragments accumulate in hyperbaric oxygen-treated lenses. The existence of such peptides in the guinea pig lens should allow investigation of the contribution of the peptides in age-related crystallin aggregation

Many of the age-related protein modifications occurring in human lenses can be replicated by incubation of lens proteins with ascorbate and oxygen [103,114–116,121,134,193]. *In vitro* studies have shown that ascorbate oxidation products are involved in these reactions [115, 193–195]. Based on these observations, it has been proposed that high concentrations of ascorbic acid and its oxidation products are responsible, in part, for the age-related changes in human lenses. However, the role of ascorbate oxidation products in lens aging cannot be tested in rodent models because these animals have very low vitamin C levels. To overcome this problem, a transgenic mouse model has been developed in which vitamin C transporter is overexpressed in lens epithelium and cortical fiber cells [110]. The lenses of these transgenic mice have high levels of vitamin C and its oxidation products. Analysis of the lenses show the accumulation of several AGEs identical to those found in aging human lens. In addition, the lenses of aged transgenic mice show the yellow discoloration characteristic of older human lenses [110,111,196]. It is yet to be determined whether the transgenic lenses show increased crystallin oxidation, proteolysis and crystallin aggregation. If they do, the mouse lens over-expressing vitamin C transporter would be a good model to study the human lens aging and to test the potential of drugs to delay the lens aging.

6. Concluding Remarks

The lens is a unique tissue with long-lived proteins, called crystallins, classified into three groups. The lens grows throughout the lifetime of an individual, and significant changes occur in the structure and function of lens crystallins. Various modifications, such as deamidation, truncation, oxidation, glycation, and methylation, lead to structural changes in the crystallins. These mechanisms play a major role in converting the largely soluble pool of crystallins into the largely insoluble pool with aging. Studies suggest that the same modifications decrease the chaperone activity of α -crystallin. Diminishing chaperone activity and altered short-range order interactions likely contribute to the protein aggregation in the lens. The inability of the lens to remove or repair the damaged crystallins results in the accumulation and aggregation of the modified proteins. Recent studies show that crystallin fragments accumulating in the lens may have a role in lens protein insolubilization. The formation of aggregates capable of light scattering is the starting point for cataractogenesis. Of the several animal models available to

study lens protein changes and cataractogenesis, hyperbaric oxygen treatment of guinea pig seems to be the only model that can replicate many of the age-related changes occurring in human lenses. The newer, 'humanized' mouse lens with ascorbate transporter appears to be a promising model to study age-related crystallin modification.

It seems that protein modification and aggregation is the hallmark of lens aging, and controlling these two events may be a key to slowing the aging process in the lens. The protein modification is directly linked to the depleting antioxidative mechanisms. It has been proposed that by midlife, a "barrier" [197] is created in human lens that impedes the free movement of antioxidant molecules from the periphery of the lens, where they are in high concentrations, to the nuclear region, where they are most needed. However, the molecular mechanism for formation of this barrier is not fully understood. Methods that would delay the formation of the "barrier" should allow the lens to remain transparent well into the later years of life. At this time it is not known how and why the crystallin fragments with anti-chaperone activity accumulate in aging lenses. The development of methods to control the anti-chaperone peptide formation should provide a means to delay the lens crystallin aggregation by prolonging the chaperone activity of α -crystallin.

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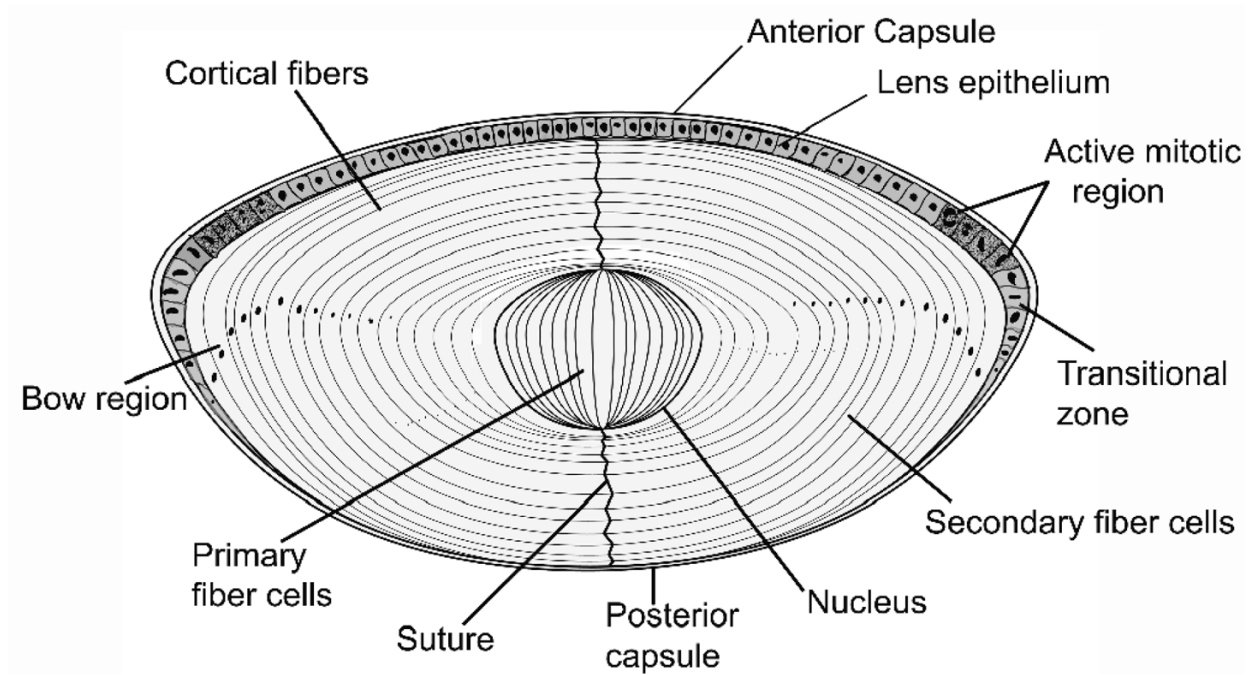


Fig. 1. A drawing of lens showing the single layer of epithelial cells covering the anterior cross section

The elongated fiber cells are directly in contact with epithelial layer in the anterior region, whereas they make contact with capsule in the posterior region. In the lens bow region the cells differentiate, elongate, lose their organelles and begin to form newly differentiated fiber cells.

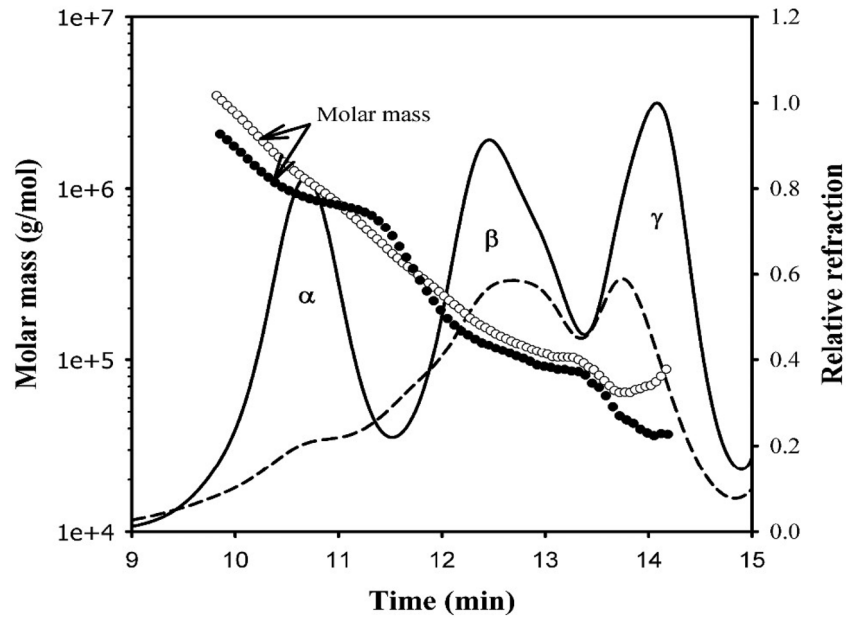


Fig. 2. Mass distribution in the water-soluble fraction of young (19 years, solid line and filled circle) and aged (83 years, broken line and open circle) human lenses using multi-angle light scattering The samples (250 mg of proteins) were injected into a TSK G5000PW_{XL} size exclusion column attached to a Shimadzu high pressure liquid chromatograph system with refractive index detector. The eluting proteins were analyzed with the help of Wyatt multi-angle light scattering and quasi-elastic light scattering detectors. The mobile phase contained 0.05 M sodium phosphate and 0.15 m sodium chloride, pH 7.2. The mass was determined using ASTRA software from Wyatt. Reproduced with permission from the J. of Biol. Chem. (2008) 283 8477–8485.

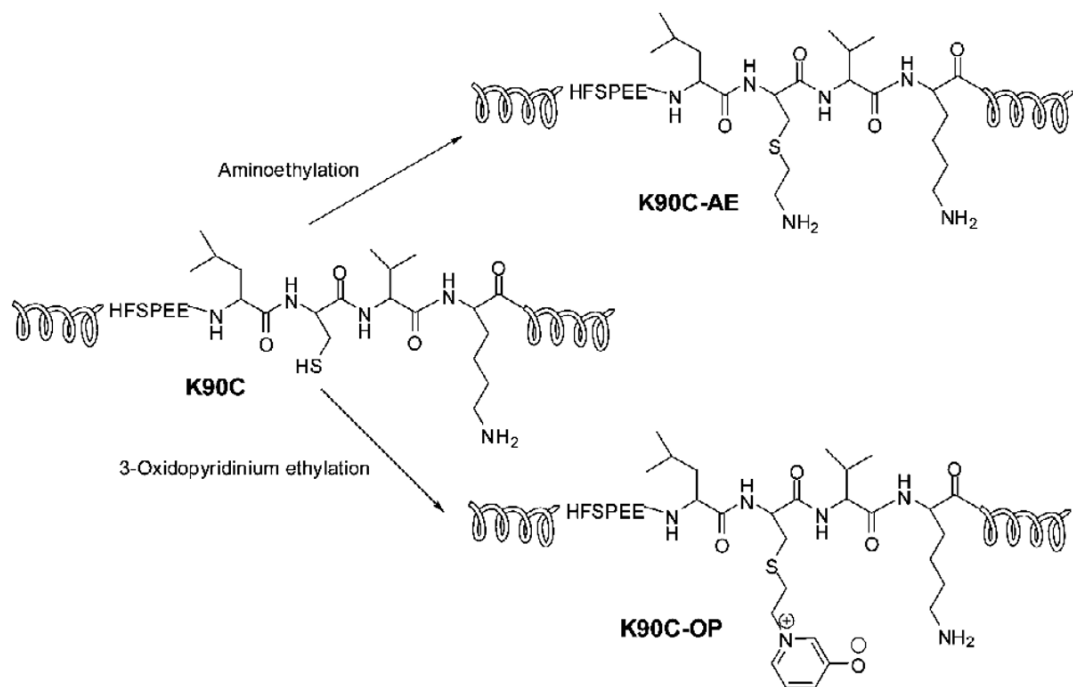


Fig. 3. Schematic representation of the modification of aB-crystallin-K90C to K90C-AE [aminoethylation] and K90C-OP [3-oxidopyridinium ethylation]
Reproduced with permission from *Biochemistry* (2008) 46, 14682–92.

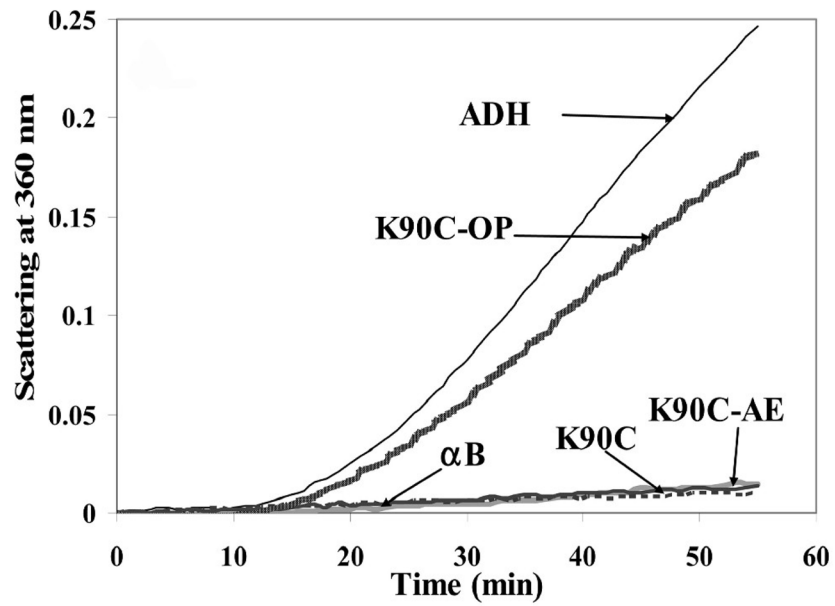


Fig. 4. Aggregation assay with wild-type α B-crystallin, K90C, K90C-AE, and K90C-OP using alcohol dehydrogenase [ADH] as client protein

The assays were carried out as described earlier [120] using 1:10 w/w ratio of α B-crystallin (native or modified) and ADH. Reproduced with permission from *Biochemistry* (2008) 46, 14682–92.

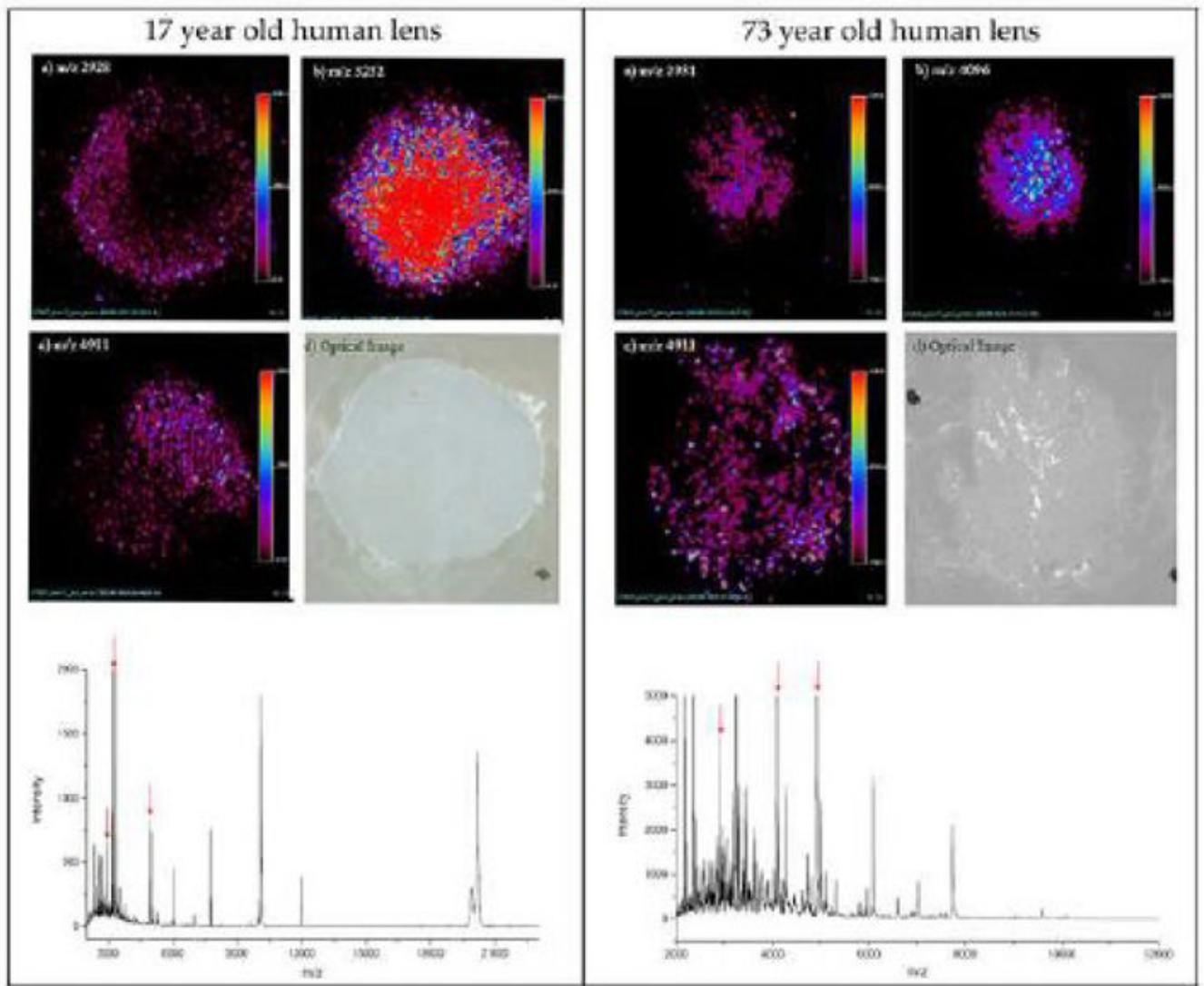


Fig. 5. MALDI imaging of a 17- and a 73-year-old human lens

The three ion images correspond to three distinct molecular species measured from a 12 micron section. The three ion images shown in 17-year-old human lens have m/z values of 2928, 3252, and 4911. The three ion images shown in 73-year-old human lens have m/z values of 2931, 4096, and 4911. The optical image [d] of the tissue specimen shown was captured following application of sinapinic acid matrix by spray coating. The data were compiled into Analyze 7.5 format after baseline correction. A 2-D ion density map (image) for specific ion constructed is shown. The procedure allows the construction of images for the specific mass signal detected from the surface of the section, theoretically offering an opportunity to generate hundreds of images, each representing the distribution of the specific ionized species from a single imaging mass spectrometry experiment. Average mass spectrum collected from the surface of the lens samples are shown in the bottom of the figure. Notice the abundance of ions in the spectrum for 73-year-old human lens. Red arrows denote the ions highlighted in figures. Data in this Figure is unpublished results from authors study.

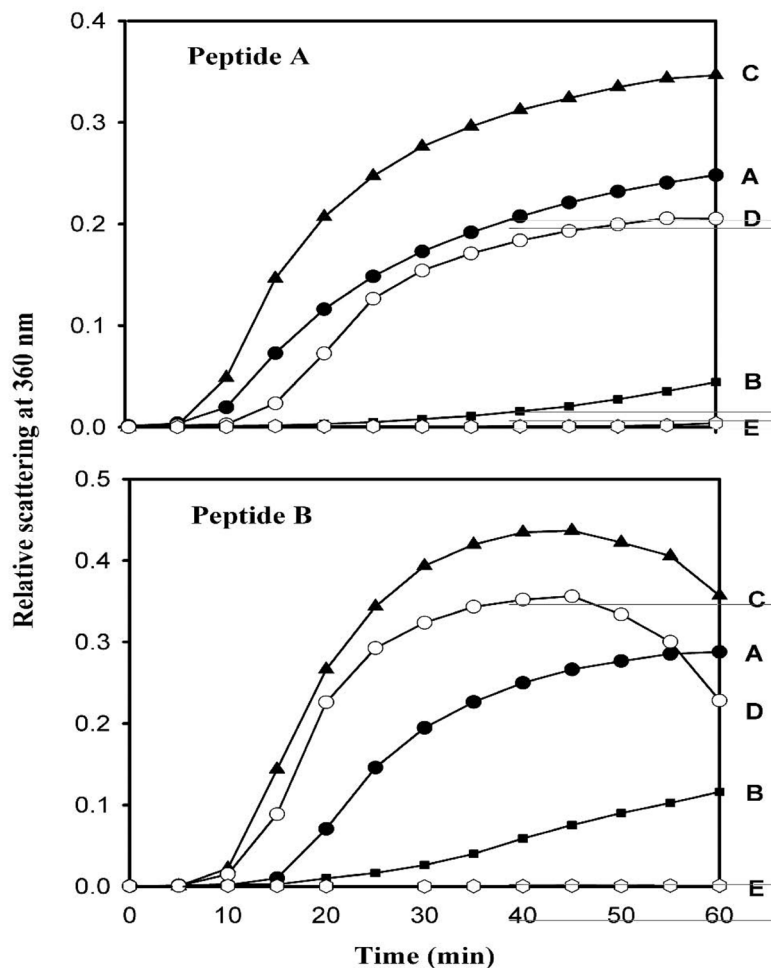


Fig. 6. Inhibition of chaperone-like activity of α -crystallin against β_L -crystallin by peptide A and B

Peptide A: αB -¹MDIAIHPWRRPFFPFH¹⁸; Peptide B: $\beta A3/A1$ ⁵⁹SNAYHIERLMSFRPIC⁷⁴Top, anti-chaperone activity of Peptide A. Bottom, anti-chaperone activity of peptide B. A, 100 mg of β_L -crystallin; B, β_L -crystallin + 25 mg of αA -crystallin; C, β_L -crystallin + 60 mg of peptide; D, β_L -crystallin + αA -crystallin + peptide; E, peptide alone (60 mg). The aggregation assay was carried out as described earlier [185]. Reproduced with permission from the Journal of Biol. Chem. (2008) 283 8477–8485.

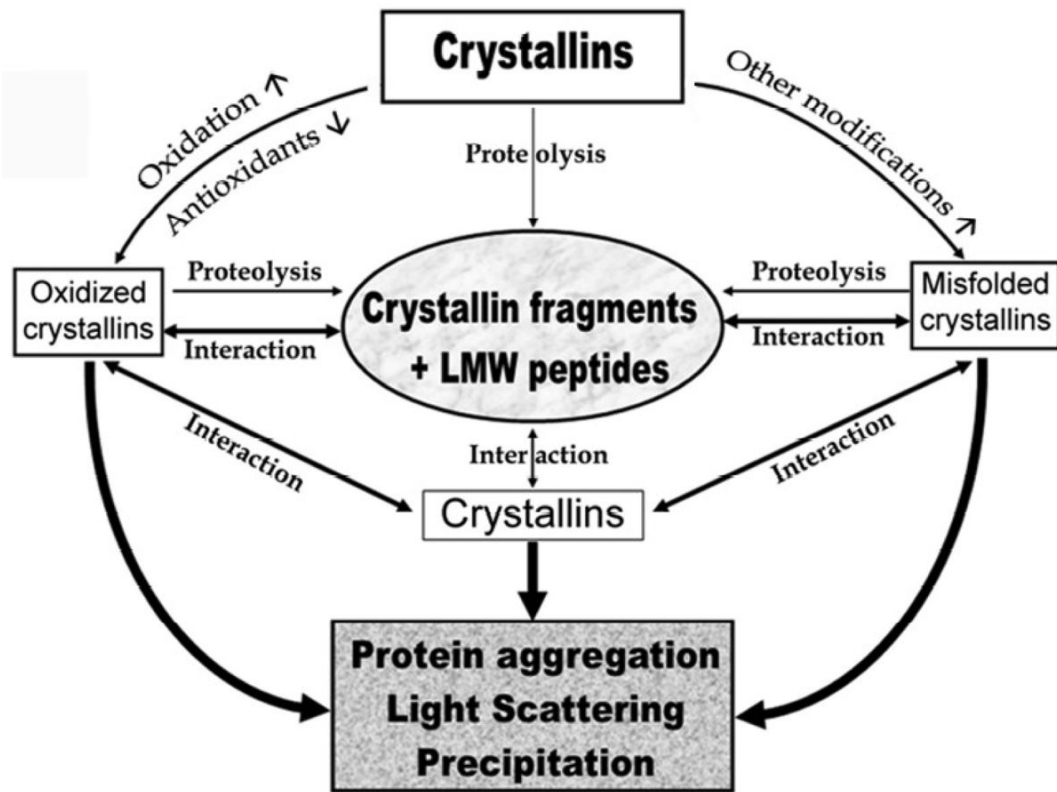


Figure 7. Schematic representation of the proposed role of crystallin fragments in lenticular aging and cataractogenesis
Oxidizing and modifying reactions/factors are not shown for simplicity.

Table 1

The Human Lens Crystallins

Crystallin	No. of Residues	Monomer [kDa]	SwissPort No
a-crystallins			
aA	173	19909	P02489
aB	175	20159	P02511
b-crystallins			
bA1	198	23191	P05813
bA2	196	21964	P53672
bA3	215	25150	P05813
bA4	195	22234	P53673
bB1	251	27892	P53674
bB2	204	23249	P43320
bB3	211	24230	P26998
g-crystallins*			
gS	177	20875	P22914
gA	173	20761	P11844
gB	174	20776	P07316
gC	173	20747	P07315
gD	173	20607	P07320

*gE and gF- are not expressed in humans whereas these genes are expressed in bovine lenses

Table 2
Post-translational/age-related modifications in human lens crystallins

Crystallin	Modification	References
aA	Deamidation Q6, Q50, Q90, N101, Q104, N123, Q147	[32–34,87,88,144,198–200]
	Oxidation M1, W9, Y18, Y34, M138, C142,	[32,33,75,84]
	Phosphorylation T13, S45, S122, T140	[32,34,75,144,199]
	Methylation R21, K88	[75]
	Acetylation M1, K70, K78, K145	[34,75,144,201–203]
	Disulphide C131–C142	[84]
	Other C131, S162, Unknown S20, H79	[34,84,144,204,205]
aB	Deamidation Q25, N78, Q108, N146	[32–35,76,144,173]
	Oxidation M1, H7, H7, W9, Y48, W60, M68,	[32–35,75,144,173]
	Phosphorylation S19, S21, S43, S45, S53, S59, S76,	[32,34,75,144,199,206]
	Methylation R22, R50	[75]
	Acetylation M1, K92,	[32–34,75,144,201,207]
	Other H83, T170, K175	[34,144,204,205,208,209]
	βA2	Phosphorylation S30
€	Acetylation S1	[34,210]
bA3/A1	Deamidation Q38, N40, Q42, N54, N62, N103, N120, N133, Q164, Q172, Q180, Q203, Q206	[33–35,102,144,173,211]
	Oxidation M46, W96, W99, M111, M126, M151, M161, W168, C52, H201	[34,35,75,84,144,173,211]
	Phosphorylation T127, S160	
	Methylation C82, C117, R137, C185	[75]
	Acetylation M1, A1[A18], K122, K125, K131,	[34,75,143,144,203] [34,75,144,201]
βA4	Deamidation Q62, Q64, Q65, N82, N113	[34,144,211]
	Oxidation M13, W79, W148	[34,211]
	Phosphorylation S34, T43	[75]
	Acetylation T1	[34,144,201]
	Other C165	[84]
bB1	Deamidation N15, N57, N67, Q105, N107, N124, Q146, N157, N161, Q196, Q204, N216, Q222, Q224, Q226, Q235	[33–35,102,144,173,211]
	Oxidation W100, M112, W123, W126, M136, W192, W215, W218, M225,	[34,75,144,211]
	Phosphorylation S9, T11,	
	Methylation R229, R230, K234	[75]
	Acetylation S1, K5, K159,	[75]
	Other S151, C79, H214	[34,75,201] [144,212]

Crystallin	Modification	References
bB2	Deamidation N115, Q146, Q154, Q162, Q182, Q184	[34,144,201,211]
	Oxidation W58, M121, W150, M192C37	[34,75,84]
	Phosphorylation T117	[75]
	Methylation K41, K67, K120	[75]
	Acetylation A1, K75, K120	[34,144,201,203,211]
bB3	Deamidation N155	[34,144]
	Oxidation M129, C39, C45	[75,84]
	Phosphorylation Y29	[213]
	Methylation K128	[75]
	Acetylation M1, A2, K128	[34,144,201]
γB	Deamidation Q66	[34,144]
	Oxidation W68, M69, M102	[34,144]
	Phosphorylation Y62, Y65	[75]
	Methylation C22, C79	[34,142]
	Carbamylation 1G	[142]
gC	Deamidation N24, Q66,	[34,144]
	Oxidation Y55, W68, M69, M101/102, W130	[34,75,144]
	Phosphorylation Y62, Y65	[75]
	Methylation C22, C79	[34,142,144]
	Carbamylation 1G	[142]
	Disulfide C22–C32, C78–C79	[33]
gD	Deamidation Q12, N49, N160	[34,144]
	Oxidation Y45, W156	[75,144]
	Methylation C110	[34,142,144]
	Carbamylation G1	[34,142]
	Disulphide C18–C32, C108–C110	[33]
gS	Deamidation N14, Q16, N53, Q63, Q70, N76, Q92, Q120, N143, Q170	[33,34,87,88,93,144]
	Oxidation M58, M73, M118, M123, M136	[33,34,75,144]
	Phosphorylation S89	[34,35,75,141,144]
	Methylation K6, C24, C26,	[35]
	Acetylation S1	[34,201]
	Disulphide C22–C24, C114–C129	[33]
	Others C82, C129, R19	[35,212]

For details on other modifications please refer to the citations. Unidentified modifications and those reported as present in low abundance are in Supplement Table 5 in reference [34].

Reference [34] is the most comprehensive analysis of age-related modification of human lens crystallins.

Table 3
Advanced glycation end products [AGE] in aging lenses

AGES	Ref
Hydroimidazolone [isomer 1, MG-H1]	[109]
Hydroimidazolone [isomer 2, MG-H2]	[109]
Hydroimidazolone [isomer 3, MG-H3]	[109]
Argpyrimidine	[108]
Tetrahydropyrimidine	[109]
N ^ε -[1-carboxyethyl]lysine [CEL]	[83,109]
Carboxymethyl lysine [CML]	[214]
Vesperlysine	[214,215]
1,3-di[N ^ε -lysino]-4-methyl-imidazolium [MOLD]	[82,109]
Glyoxal lysine dimer [GOLD]	[82,113]
Pentosidine	[80]
1-(5-Amino-5-carboxypentyl)-4-(5-amino-5-carboxypentylamino)-3-hydroxy-2,3-dihydropyridinium (K2P)	[216]
N ^ε -Fructosyl-lysine	[217]
Furosine	[214]
Pyrraline	[105]
2-Ammonio-6-(3-oxidopyridinium-1-yl)hexanoate (OP-lysine)	[121]
Glucosepane	[213]

For structures of these AGE's see the references

Table 4
Effect of truncation on the oligomeric mass of aA-crystallin

	Molar mass at the peak apex Mw [g/mol]	Hydrodynamic radius
a A	$7.50 \pm 0.02 e^5$	8.0 ± 0.2
aA: a A ₁₅₁ [3:1]	$14.0 \pm 0.04 e^5$	9.6 ± 0.2
aA: a A ₁₅₁ [2:1]	$20.0 \pm 0.01 e^5$	11.8 ± 0.3