Effect of aging on the chaperone-like function of human α -crystallin assessed by three methods

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α -Crystallin can function as a molecular chaperone by preventing unwanted interactions. This paper presents the effects of aging and cataract on the chaperone-like properties of α -crystallin from soluble fractions from the cortex and nucleus of human lenses by using three assays: enzyme inactivation and two turbidity experiments. The three methods complemented each other. There was no decrease with age of chaperone-like function

of cortical α -low and α -high crystallin. Nuclear α -low crystallin showed a decrease, whereas α -high crystallin showed no age-related change but its protective effect was diminished. Results from the nucleus of 40-year-old cataractous lenses seemed similar to those for clear lenses of equivalent age, whereas 80-year-old cataractous lenses showed decreased chaperone-like behaviour.

INTRODUCTION

The crystallins are major structural proteins within the lens that account for up to 90 % of total soluble protein. There are three distinct families: α -, β - and γ -crystallin. α -Crystallin contributes approx. 40 % of total lens structural protein mass and consists of two types of 20 kDa subunit, α A and α B, exhibiting 57 % similarity [1]. These subunits non-covalently self-associate to form a large physically heterogeneous macromolecular complex with an average molecular mass of approx. 800 kDa. In addition there is a larger aggregate of high molecular weight, α -high, which contains small amounts of β/γ -crystallins and other proteins [2]. α -High and α -low crystallins have mainly the same subunits [3]. CD measurements provide strong evidence that the secondary structure of α -crystallin is mainly β -pleated [4]. The tertiary and quaternary structures have not yet been elucidated. Recently α A- and α B-crystallins have been shown to exist outside the lens in organs such as brain, spleen and heart [5,6]. Elevated levels of α B-crystallin have been found in various pathological conditions [7,8] as well as in neurodegenerative disorders [9–11]. It is not certain what role α -crystallin has in these conditions.

In the central part of the lens there is no measurable protein synthesis and no renewal of damaged proteins [12]. New cells from the epithelial monolayer contribute to lens growth by building up layer after layer around an original nucleus. No cells are lost and therefore nuclear proteins have existed from birth. Post-translational modifications (PTM) occur to α -crystallin with increasing age and during cataract formation. Aggregation of lens proteins produced by these PTM would disturb the short-range organization of the crystallins necessary for transparency [13]; if of sufficient size it would lead to light scattering and cataract.

Ingolia and Craig [14] identified a startling homology between an α -crystallin polypeptide sequence and those of the small heat-shock proteins from *Drosophila*. The most characterized heat-shock protein is GroEL from *Escherichia coli*, which prevents unwanted aggregation of newly formed polypeptides and assists in the refolding of a wide variety of different proteins without being directly involved with their biological function, acting like a molecular chaperone [15,16]. Horwitz suggested that α -crystallin could act in a chaperone-like manner after showing its

suppression of thermal aggregation of enzymes and lens β/γ -crystallins [17]. Many other methods of assaying α -crystallin have shown chaperone-like ability. The molecular mechanism of the interaction between α -crystallin and its substrates remains unknown. Horwitz et al., with bovine α -crystallin, reported a decrease in chaperone activity from calf cortical α -low crystallin and 5-year-old nuclear α -low crystallin by using the thermal aggregation of β -low crystallin in the assay [18]. Cherian and Abraham [19] showed a loss of chaperone-like activity of human α -crystallin from years 1–20 and 70–79 by using only two groups of α -low crystallin from whole lenses.

We investigated the effects of aging on the chaperone-like ability of α -crystallin from clear and cataractous lenses. The α -high and α -low crystallin fractions were isolated from cortex and nucleus and their chaperoning ability was investigated by three independent methods. These included sugar inactivation as well as thermal aggregation of β -low crystallin and of malate dehydrogenase (MDH).

MATERIALS AND METHODS

Materials

NADH, oxaloacetate and MDH (EC 1.1.1.37) were purchased from Sigma Chemical Co. (Poole, Dorset, U.K.). Sephacryl S-300HR was purchased from Pharmacia Ltd (Milton Keynes, Bucks., U.K.). Clear human lenses (7 months and 5, 20, 40 and 80 ± 2 years) were from donor eyes from Oxford and Bristol eye banks. Cataractous lenses (40 and 80 ± 2 years, nuclei only; Grade II Pirie Scale) were from the Oxford Eye Hospital.

Isolation and purification of crystallins

Frozen lenses were thawed and separated into cortex and nucleus. For young lenses large forceps were inserted at the edge of the nucleus and the cortex was scraped away with a scalpel. For older lenses, gentle rubbing between thumb and index finger sufficed to remove the cortex from the physically larger nucleus. Lenses were pooled (7 months, $n = 2$; 5 years, $n = 4$; 20 years, $n = 6$; 40 years, $n = 6$; 80 years, $n = 6$; 40 years cataract, $n = 4$; 80

Abbreviations used: AGE, advanced glycation end-product; MDH, malate dehydrogenase; PTM, post-translational modifications.

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Figure 1 SDS/PAGE analysis of α -crystallin preparations

Lane M, marker proteins (from the top: ovotransferrin, 76–78 kDa; albumin, 66 kDa; ovalbumin, 42 kDa; carbonic anhydrase, 30 kDa; myoglobin, 17 kDa; cytochrome *c*, 12 kDa); lane 1, cortical α -low crystallin; lane 2, cortical α -high crystallin; lane 3, nuclear α -low crystallin; lane 4, nuclear α -high crystallin; lane 5, cataractous nuclear α -low crystallin; lane 6, cataractous nuclear α -high crystallin. α -Crystallin sources: (a) 7-month-old lenses; (b) 5-year-old lenses; (c) 20-year-old lenses; (d) 40-year-old lenses; (e) 80-year-old lenses.

years cataract, $n = 6$). The fractions were then weighed and homogenized in seven times their weight of 0.05 M sodium phosphate buffer, pH 6.7. The suspension was centrifuged at 4 °C for 40 mins at 22440 *g*. The supernatant was applied to a Sephacryl S-300HR size-exclusion gel-chromatography column (2.3 cm $\times$ 100 cm) and eluted with the aforementioned buffer at a flow rate of 25 ml/h [20]. Fraction volumes of approx. 4 ml were collected. The fractions corresponding to α -high, α -low, β -high, β -low and γ -crystallin were pooled separately and dialysed against water with three changes of water over 24 h. The pooled fractions were freeze-dried separately, their purity was checked by SDS/PAGE and they were stored at -20 °C until required. Further purification on Sephacryl S-500 HR column of α -crystallin did not change results, so Sephacryl S-300 HR was used for fractionating the crystallins mentioned.

MDH assay

The enzyme activity of MDH from pig heart mitochondria was determined by monitoring the decrease in absorbance of NADH at 340 nm as it is oxidized by oxaloacetate [21], with a Kontron 930 spectrophotometer. All solutions were prepared in 0.05 M potassium phosphate buffer, pH 7.5, and sterilized through a 0.2 μ m pore-size Millipore filter. MDH (75 μ mol in 30 μ l) was incubated in the spectrophotometer at 37 °C in a quartz cuvette

with 2.84 ml of the above buffer (preheated to 37 °C), 100 μ l of oxaloacetate (2 mg/ml) and 30 μ l of NADH (10 mg/ml). The mixture was stirred rapidly and the decrease in absorbance was monitored over 1 min against a buffer blank.

Incubation with sugar

Fructose was used as it inactivated the enzyme MDH more quickly than glucose or glucose 6-phosphate [21]. MDH was incubated with 5 mM fructose in 0.05 M potassium phosphate buffer, pH 7. Stock solutions of the incubation solution with and without the sugars were made and separated into 1.5 ml sterile vials with rubber tops. The vials were placed in a shaking water bath at 37 °C. Zero time readings were taken in triplicate from single vials and further vials were assayed at 2, 4 and 6 h.

Protective effects of α -crystallin against inactivation by sugar

α -Crystallin has been shown to protect MDH against glycation-induced inactivation [21]. α -Crystallin was dissolved in 0.05 M potassium phosphate buffer, pH 7.0, and added to the solution of enzyme to give final concentrations of 1.2, 0.12 and 0.06 mg/ml in the incubation, with and without fructose, corresponding to MDH-to- α -crystallin ratios of 1:0.1, 1:0.01 and 1:0.005 (w/w). The incubation solutions were made up in 1.5 ml sterile glass

vials as above, and incubated as above. Chaperone-like ability was represented as the percentage activity remaining compared with the corresponding incubation with α -crystallin control.

β -Low crystallin aggregation assay

Heat-induced aggregation of 3-month-old rabbit β -low crystallin was performed as described by Horwitz [17]. A stock of 1.87 mg of β -low crystallin was weighed and dissolved in 6 ml of 0.05 M sodium phosphate buffer, pH 6.7 (degassed before dissolving). Taking 0.8 ml gave a concentration of 250 μ g/ml target protein for five cuvettes. A stock of 1.25 mg/ml α -crystallin was dissolved and 200–20 μ l taken so as to give a final incubation concentration of 250–25 μ g/ml [α -crystallin-to- β -low crystallin ratios of 1:1, 1:2.5, 1:5 and 1:10 (w/w)] in 1 ml of the aforementioned buffer in plastic cuvettes. Two controls, β -low crystallin and the α -crystallin under observation, were examined in every experiment. The cuvettes were heated at 55 °C for the aggregation of β -low crystallin. The attenuation, i.e. the scattering resulting from aggregation, was measured continuously for 60 min spectrophotometrically at 360 nm. The data from the spectrophotometer were stored on computer disks for off-line analysis. Chaperone-like protection was represented as the percentage of prevention by α -crystallin of the scattering produced by the target protein control after 60 min.

MDH aggregation assay

Heat-induced aggregation of MDH closely followed that described above. A solution of MDH (10.4 mg/ml protein) was diluted with buffer; 0.8 ml of this was taken to give a final concentration of 250 μ g/ml in the cuvettes. A stock of 1.25 mg/ml α -crystallin was dissolved and 200–20 μ l taken so as to give a final incubation concentration of 250–25 μ g/ml [α -crystallin-to-MDH ratios of 1:1, 1:2.5, 1:5 and 1:10 (w/w)] in 1 ml of the aforementioned buffer in plastic cuvettes. The cuvettes were heated at 50 °C and the attenuation was measured for 60 min as above. Again, two controls, MDH and the α -crystallin under observation, were examined in every experiment.

Electrophoresis

SDS/PAGE [12.5 % (w/v) gel] was performed as described by Laemmli [22] under reducing conditions with LKB 2052 Midget Electrophoresis Units. Protein (1 mg/ml) was dissolved in the sample buffer containing 5 % (v/v) 2-mercaptoethanol. Coomassie Brilliant Blue G was used to detect the polypeptide bands.

Analysis

Statistical analysis was performed with Student's *t* test.

RESULTS

The purity of the α -crystallin fractions was confirmed with SDS/PAGE, although during aging and cataract formation the two α -crystallin subunit bands become slightly diffuse (Figure 1). This is more prominent with the nuclear fractions, which also contain several polypeptides with higher molecular masses (corresponding to β -crystallins). These polypeptides probably represent less than 10 % of the α -crystallin preparations except in the 80-year-old lens. The elution profiles from the clear soluble protein from human lens for the nuclear and cortical fractions both show a gradual decrease of the α -low crystallin peak and concurrently an increase in the α -high crystallin peak (results not

Figure 2 Three assays for chaperone activity

Top panel: fructation (5 mM) of MDH and protection by α -crystallin; inhibition of MDH without α -crystallin (■), with the addition of 1.2 mg/ml α -crystallin (●) and with the addition of 0.06 mg/ml α -crystallin (○). Middle panel: Increase in turbidity of β -low crystallin (250 μ g) with and without α -crystallin. The numbers adjacent to the curves represent the amount of added α -crystallin (in μ g). Bottom panel: increase in turbidity of MDH (250 μ g) with and without α -crystallin during heating. The numbers adjacent to the curves represent the amount of added α -crystallin (in μ g).

shown), as has been reported by others [1]. The 80-year-old grade 2 cataract lenses showed a marked decrease in α -low crystallin and increased amounts of α -high crystallin.

Inactivation of MDH with 5 mM fructose was complete within 6 h, suggesting non-enzymic binding to the enzyme molecule (Figure 2, top panel). Addition of 1.2 mg/ml α -crystallin main-

Table 1 Chaperone-like activity of cortical α -crystallins determined by three methods

The cortex was separated from the nucleus of clear lenses and fractionated. The assays were: protection against the fructose-induced inactivation of MDH by α -crystallin [MDH-to- α -crystallin ratio 100:1 (w/w)], protection of heat-induced aggregation of β -low crystallin (250 μ g) by α -crystallin (100 μ g), and protection of heat-induced aggregation of MDH (250 μ g) by α -crystallin (100 μ g). Values are means $\pm$ S.E.M.; for *n* values see the Materials and methods section.

Crystallin type	Age (years)	Activity remaining after enzyme inactivation (%)	Protection against β -low aggregation (%)	Protection against MDH aggregation (%)
Cortical α -low	0.58	90.53 $\pm$ 3.53	91.14 $\pm$ 1.455	52.0 $\pm$ 2.63
	5	80.33 $\pm$ 2.1	91.54 $\pm$ 0.93	40.55 $\pm$ 3.78
	20	74.8 $\pm$ 5.7	92.54 $\pm$ 1.213	42.36 $\pm$ 6.7
	40	74.66 $\pm$ 3.53	80.33 $\pm$ 14.02	40.46 $\pm$ 5.98
	80	70.02 $\pm$ 7.8	81.2 $\pm$ 7.39	37.67 $\pm$ 2.35
Cortical α -high	0.58	77.0 $\pm$ 8.21	89.8 $\pm$ 8.75	33.2 $\pm$ 1.34
	5	79.33 $\pm$ 3.57	84.04 $\pm$ 7.29	34.2 $\pm$ 2.8
	20	76.2 $\pm$ 5.14	78.78 $\pm$ 2.84	31.2 $\pm$ 4.17
	40	76.2 $\pm$ 6.04	74.73 $\pm$ 3.47	28.2 $\pm$ 6.96
	80	71.87 $\pm$ 6.37	74.05 $\pm$ 3.13	32.0 $\pm$ 0.12

Table 2 Chaperone-like activity of nuclear α -crystallins determined by three methods

The nucleus was separated from the cortex from clear and grade II cataract (Cat) lenses and fractionated. For assays see Table 1. Values are means $\pm$ S.E.M.

Crystallin type	Age (years)	Activity remaining after enzyme inactivation (%)	Protection against β -low aggregation (%)	Protection against MDH aggregation (%)
Nuclear α -low	0.58	92.0 $\pm$ 4.61	93.17 $\pm$ 0.29	47.46 $\pm$ 7.31
	5	87.0 $\pm$ 11.96	77.27 $\pm$ 4.67	34.77 $\pm$ 2.9
	20	73.66 $\pm$ 8.25	73.41 $\pm$ 4.46	34.15 $\pm$ 4.21
	40	71.0 $\pm$ 7.25	72.92 $\pm$ 6.42	33.27 $\pm$ 3.27
	40 (Cat)	69.33 $\pm$ 3.66	48.98 $\pm$ 1.43	34.8 $\pm$ 6.65
	80	61.7 $\pm$ 1.65	66.71 $\pm$ 3.87	36.07 $\pm$ 4.7
	80 (Cat)	59.86 $\pm$ 6.37	16.3 $\pm$ 3.87	17.3 $\pm$ 1.09
Nuclear α -high	0.58	56.0 $\pm$ 2.47	26.15 $\pm$ 2.45	16.67 $\pm$ 0.66
	5	54.2 $\pm$ 1.01	26.84 $\pm$ 2.13	17.15 $\pm$ 1.71
	20	60.2 $\pm$ 4.9	31.43 $\pm$ 2.45	16.44 $\pm$ 4.34
	40	53.66 $\pm$ 6.35	32.35 $\pm$ 4.07	14.22 $\pm$ 1.08
	40 (Cat)	59.63 $\pm$ 6.82	21.2 $\pm$ 3.12	14.67 $\pm$ 2.73
	80	47.9 $\pm$ 12.76	29.5 $\pm$ 5.04	15.55 $\pm$ 5.50
	80 (Cat)	33.6 $\pm$ 3.15	4.05 $\pm$ 2.21	7.125 $\pm$ 1.34

tained maximum activity, whereas 0.06 mg/ml α -crystallin offered only 25% protection from inactivation. The protective effect was not due to preferential glycation of α -crystallin over the enzyme, as proteins such as lysozyme, which has a similar lysine content, and BSA, which has a greater lysine content, displayed no such protective ability [21]. Scattering due to β -low crystallin aggregation occurred from 10 min (Figure 2, middle panel). Addition of 250 μ g/ml α -crystallin to a solution containing 250 μ g/ml β -low crystallin completely protected the β -low crystallin, whereas 100 μ g/ml gave 90% protection. Similar results were seen for heat-induced aggregation of MDH except that 100 μ g/ml α -crystallin gave only 50% protection (Figure 2, bottom panel). The addition of lysozyme or BSA to the target protein offered no protection from aggregation. The period of 60 min was chosen for the calculation of protective effects. Further incubation caused the protein to aggregate to such a size as to come out of solution and fall to the bottom of the cuvette, causing a decrease in light scattering.

The results for the inactivation of MDH by fructation assay are shown in Tables 1 and 2. There was no statistically significant decrease in chaperone-like activity of cortical α -low and α -high crystallin with age. In contrast, nuclear α -low crystallin showed a gradual age-dependent loss in chaperone-like ability of approx. 30% ($P = 0.003$). The nuclear α -high crystallins showed no age-

dependent loss in chaperone-like activity but showed approx. 30% less activity than the young cortical α -low crystallin. Only the nuclear fractions were investigated from the cataractous lenses owing to the preferred method of extraction by the surgeons. Curiously, α -low crystallin from grade II cataracts showed no difference from their corresponding clear lenses in chaperoning ability. The 40-year-old cataractous lenses showed the same amount of protection as the α -high fraction from 40-year-old clear lenses, but the α -high crystallin from the 80-year-old cataract lenses had 30% less protective effect than the corresponding clear lenses.

The results for the thermal aggregation of β -low crystallin assay are shown in Tables 1 and 2. The pattern is very similar to that of the previous method. There was no loss of protection against aggregation with cortical α -low or α -high crystallin with age ($P = 0.766$). Seven-month-old nuclear α -low crystallin fraction showed the same protection as cortical α -low crystallin, but there was a gradual age-dependent decrease in protection by nuclear α -low crystallin ($P = 0.001$). Nuclear α -high crystallin showed approx. 50–60% ($P < 0.001$) less activity than the young nuclear α -low crystallin but showed no further significant changes in chaperone-like ability with age (Table 2). As stated above, only nuclear fractions were taken from cataractous lenses. There was an age-dependent loss of approximately 30% ($P < 0.001$) in

cataract nucleus α -low crystallin between the two ages. Both ages showed decreased chaperone-like protection compared with their respective clear-lens counterparts. α -High crystallin from the nucleus of cataractous lenses showed an age-dependent loss in chaperone-like ability of approx. 25 % ($P = 0.013$) of an already compromised chaperone. The α -high crystallin from 80-year-old cataracts acted as a very poor chaperone.

The results of the assay with thermal aggregation of MDH are shown in Tables 1 and 2, and show a similar pattern to the two methods above, although α -crystallin protected β -low crystallin more than it did MDH. There was no loss in the chaperone-like protection of cortical α -low ($P = 0.283$) and α -high crystallin with age. The nuclear α -low crystallin showed an early decrease in chaperone-like ability between 7 months and 5 years, after which no decrease with age was observed. Again the nuclear α -high crystallin showed compromised protection against aggregation but no change with age. The nuclear α -low and α -high crystallin from grade II cataracts aged 40 years showed no difference from the corresponding clear-lens results, but the 80-year-old fractions showed decreases rendering them very poor chaperones.

DISCUSSION

A previously reported decrease in the chaperone-like ability of human α -crystallin with age [19] used whole lens fractions of α -low crystallin and pooled ages from 1 to 18 years and from 70 to 79 years, making comparisons difficult. The present results indicate that the decline would have been present only in the lens nucleus.

Although lens α -crystallin, especially the low fraction, functions effectively in a chaperone-like manner, the amount of α -crystallin in the soluble fraction decreases with age, especially in the nucleus [23] and to a greater extent in cataractous lenses [24]. The water-insoluble fraction is heterogeneous and amounts vary widely, so it gives little clue to the percentage of the total α -crystallin being measured. α -High crystallin has been considered to be an intermediate between the soluble and insoluble fractions. It is also plausible that α -high crystallin is partly a permanent chaperone complex unable to protect more proteins. The result of aging is the decrease in active α -crystallin to provide protection.

These consistent observations with three assays support the theory that α -crystallin can act as a molecular chaperone. Like the bacterial chaperone GroEL [15], the C-terminal sequence is the most conserved region of the α -crystallin/small heat-shock protein family [25], and is required for chaperoning ability [26]. α -Crystallin can only recognize and bind to non-native proteins that occur very early on the denaturation pathway [27] but has apparently a very low affinity for folding intermediates formed during protein refolding reactions *in vitro*, except for claims by Jakob et al. [28]. Whatever the mechanism of the chaperone-like properties of α -crystallin it will have to explain the protection against thermal aggregation and against glycation-induced loss of enzyme activity.

C-terminal PTM of human α A-crystallin during aging *in vivo* [29] occur rapidly within the first 20 years. After this period there are no significant modifications with increasing age. Heat denaturation experiments [26] of α A-crystallin after limited tryptic digestion show a decrease in the ability to protect proteins *in vitro*, suggesting the importance of the C-terminal region; this might explain the decrease seen in nuclear α -low crystallin samples between the ages of 7 months and 20 years. The compromised chaperone-like activity of the nuclear α -high fraction might be due to the decrease in subunits possessing the C-terminal region, either by cleavage or involvement in aggregation.

The cortical fractions maintain their chaperoning ability with age. Only the peripheral cortex maintains its synthetic capabilities, having retained its organelles. Although the proteins have had less time to accumulate any damage, any occurrence of protein disruption is prevented by defence systems as well as the chaperone-like effect of α -crystallin. Scavengers of reactive oxygen species, such as glutathione, superoxide dismutase, thiol-reductase, catalase and thioltransferase, are present [30].

The effectiveness of these defence systems decreases towards the nucleus and with age [31]. The absence of protein turnover in the nucleus [12] and the layering effect of lens growth leaves the central cells remote from nutrient supply, making these long-lived proteins vulnerable. PTM seen during aging include the accumulation of α -high and water-insoluble (urea-soluble) proteins [23,24], an increased formation of chromophores and fluorophores greatest in the α -high fraction [32] and non-enzymic modifications including glycation, carbamylation, deamidation, methionine oxidation, degradation and phosphorylation, which all produce anodic shifts on isoelectric focusing [33]. Thiol oxidation and formation of disulphide bonds [34], although there are few protein-protein disulphide bonds in clear human lenses [35], and racemization of amino acids do not produce such shifts but increase with age [36]. Identification of the effect of these PTM on the chaperone-like ability of α -crystallin *in vitro* primarily used the heat denaturation assay. Cherian and Abraham [19] glycated α -crystallin with both ascorbic acid and erythrose and observed a decrease in chaperone-like ability, whereas van Boekel and others [37] showed that neither fructose nor glucose 6-phosphate caused any decrease in chaperone-like activity. Incubation with ribose caused cross-linking and loss of chaperone activity. Blakytyn and Harding [38], using an enzyme inactivation assay, showed that glycated human α -crystallin did not lose its chaperone-like ability. The extent of glycation increases markedly with age, as glucose intolerance increases with age [39]. Early glycation forms a reversible Schiff base with a free amino group. Rearrangement forms the more stable Amadori product, which can then lead to the formation of irreversible chemical modifications of proteins known as advanced glycation end-products (AGEs) [40]. These products contribute to the age-related increase in brown colour, fluorescence and insolubilization of the lens crystallins and are therefore linked to cataract. Carbamylation of α -crystallin did not alter its chaperone-like ability with the thermal denaturation assay [37,41]. Oxidative modification [37] and mixed disulphide [19] formation have a negative effect on chaperone-like activity, although there is no disulphide accumulation in clear human lenses with age, at least up to 65 years [42]. Although the function of phosphorylation is undecided, it has been shown that its effect on α -crystallin is positive compared with its non-phosphorylated form [37]. The combined effect of PTM might contribute to α -crystallin's decreased chaperone-like function, although the amount of modification needed to form the α -high fraction is not known.

Cataract can be seen as accelerated aging. The changes to crystallins are more severe than those of natural aging. Again, the defence systems decrease during cataract formation, with the inactivation of scavenging enzymes already mentioned [30]. During cataractogenesis, the lens proteins unfold and the thiols can then react to form both mixed disulphides, with glutathione and cysteine, and disulphide cross-linking aggregates in the lens [12]. PTM of lens proteins in cataract include those mentioned in aging [33] and AGE accumulation [43]. Increasing amounts of α B-crystallin were found to be expressed in brains with Alzheimer's disease [11] and might be connected with amyloid deposition and neurofibrillary tangles, both of which are rich in

histochemically detectable AGEs [44]. Glycation and AGE formation of β -amyloid protein also promotes the nucleation and precipitation of this protein. α B-Crystallin in Alzheimer's disease might have a protective role as a chaperone. Different methods of lens opacification *in vitro* and *in vivo* might have different effects on the chaperone-like activity of α -crystallin. These methods of cataract induction include sugar, radiation, steroid, selenite, cyanate and calcium, and these agents can act synergistically. It seems likely that most mechanisms of cataract formation act through a common pathway and therefore affect α -crystallin in a similar manner.

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