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Eye lens protein α-crystallin and cataract – A Review

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The lens is a transparent, biconvex structure in the eye that, along with the cornea, helps to refract light to be focused on the retina. Crystallins are water-soluble proteins that compose over 90% of the protein within the lens. The three main crystallin proteins found in the human eye lens are α-, β- and γ-crystallins. Crystallins tend to form soluble, high-molecular weight aggregates that pack tightly in lens fibers. α-Crystallin is the major structural protein and plays a dominant role in maintaining the transparency and refractive properties of the eye lens. It consists of two highly homologous subunits αA- and αB-crystallin, having molecular mass ~20 kDa each. α-Crystallin belongs to the family of small heat shock proteins and acts as molecular chaperone by preventing stress-induced protein aggregation. With age, α-crystallin forms large aggregates and its ability to prevent aggregation is lost. So transparency of the lens is disturbed and cataract may result. Cataract is the opacity or clouding of lens in the eye which leads to a decrease in vision. Cataracts usually develop as the aging lens becomes more and more opaque, but cataracts can also form congenitally or after injury to the lens. Diabetes is a risk factor for cataract. Cataract is still one of the major causes of adult blindness. Extensive research is going on to prevent cataract. The development of nonsurgical treatments is crucial for preventing or reversing cataract. The research on nanotechnology can provide suitable methodology for drug delivery to the eye.

Keywords: Eye lens, α-crystallin, oligomeric structure, chaperone activity, denaturation, hydrophobic sites, cataract, mutation, glycation, deamidation.

1. Introduction

The lens is composed of transparent, flexible tissue and is located directly behind the iris. It is the second part of our eye, after the cornea that helps to focus light and images on our retina[1]. The retina helps to transform these light rays into electric impulses which travel down the optic nerve and to the brain, the brain then formulates an image based on these impulses[2]. Eye lens is composed of 65% water and 35% proteins[3]. The main constituents of the mammalian eye lens fiber cells are crystallin proteins namely α-, β- and γ-crystallin[4]. In the eye lens of vertebrates crystallins constitute ~90% of lens protein mass[5]. Among all the crystallins, α-crystallin is the major structural protein and plays a dominant role in maintaining the transparency and refractive properties of the eye lens[1,6,7]. Birds and reptiles contain another major lens protein called δ-crystallin[8,9]. α-Crystallin alone is ~45% of total protein.

α-Crystallin consists of two highly homologous subunits αA- and αB-crystallin, having molecular mass ~20 kDa each[3,6]. In humans, αA-crystallin has 173 and αB-crystallin has 175 amino acid residues[10]. α-Crystallin is a large oligomer of 800 kDa average molecular weight having αA and αB in approx 3:1 ratio in human lens[4]. There is about 57% amino acid sequence homology between αA- and αB-crystallin[11]. α-Crystallin belongs to the family of small heat shock proteins (sHSPs) and is evolutionarily conserved from bacteria to humans[10,12–15]. It is the major protein of vertebrate eye lens, although its presence in other organs such as brain, heart, kidney, spleen, thymus etc. is known[16–19].

αB-Crystallin has a heat shock element and is induced by various stress conditions such as heat shock or oxidative stress[13,20]. It is also implicated in a number of neurological disorders such as Alzheimer's disease, Parkinson's disease etc.[16–18]. αÁ-Crystallin, on the other hand, is not stress inducible though both αA- and αB-crystallin can confer cellular thermoreistance[21,22]. Subunit masses of other members of the sHSP family range from 12–43 kDa[10]. sHSPs from prokaryotes and plants differ significantly from their mammalian counterparts[11]. Sequence analysis of sHSPs shows the presence of a highly conserved 'α-crystallin domain' of

about 100 amino acids. The sequence and length of the N- and C-terminal regions vary considerably from species to species[23,24].

While sHSPs are abundant in both eukaryotic and prokaryotic organisms, no obvious function of these proteins were known, till recently, when it has been shown that all sHSPs including α-crystallin are molecular chaperones[6,25–31]. These proteins prevent the aggregation of other proteins affected by heat or other stress conditions. α-Crystallin is known to prevent thermal aggregation[28,32], disulphide bond cleavage induced aggregation[27,33], and UV-ray induced aggregation of substrate proteins[30,34]. This function of α-crystallin is believed to play a significant role in the prevention of cataract in the ocular lens. Function of sHSPs in the non-lenticular tissues is not clear, but it is believed to play an important damage control function by maintaining substrate proteins in a folding competent environment[35]. Not much is known about the molecular structure of these proteins.

A known but striking feature common to most sHSPs and other molecular chaperones is their ability to assemble into large micelle-like oligomeric complexes[10,36–39]. In lens, αA- and αB-crystallin exist as polydisperse heteroaggregates of average molecular mass of ~800 kDa[3,6,28]. Homoaggregates of recombinant αA- and αB-crystallins grow up to ~650 kDa[40]. HSP 16.5, whose crystal structure is known, formed a 24-subunit assembly of approximately 400 kDa[41]. Electron micrographic images of HSP 26 and HSP 27 revealed spherical multisubunit assembly[42,43]. Many other known molecular chaperones such as GroEL, TriC also have large oligomeric structure[37,44]. Based on this information, oligomerization is thought to be prerequisite for chaperone function. However mechanistic details of such a correlation are poorly understood.

α-Crystallin plays a major role in maintaining the transparency of the lens by preventing stress-induced protein aggregation. With age, α-crystallin forms large aggregates and its ability to prevent aggregation is lost. So transparency of the lens is disturbed and cataract may result. Cataract can have many causes. Cataract is still one of the major causes of adult blindness. Extensive research is ongoing to prevent blindness.

2. Structure of α-crystallin

Crystallization attempts of both the natural and recombinant α-crystallin of vertebrate were not fruitful so far because of the polydispersed nature of the protein oligomer. The detailed 3D structure of the subunit and also the topology of the oligomeric assembly are not known. Cryo-electron microscopic data indicates that recombinant human αB-crystallin assembly consists mainly of protein shells of ~19 nm diameter with a diameter of ~8 nm in central cavity[45].

2.1. Primary structure

Primary structure of α-crystallin is known for many years. There is about 75% amino acid sequence homology between human and bovine α-crystallin[46,47]. Many other mammalian species have been identified with amino acid sequence homology of α-crystallin A-chain[48–50]. The amino acid sequence homology of α-crystallin A- and B-chain of different species is shown in Fig. 1. The sequence of α-crystallin is divided into three regions. (1) N-termianl region, containing ~1–60, (2) α-crystallin domain containing ~61–145 and (3) C-terminal extension containing ~146–173 (for αA-crystallin) or ~146–175 (for αB-crystallin) amino acid residues. The term "α-HSP" is meant for the small heat shock proteins (sHSPs) that contain the characteristic α-crystallin domain[51]. A common feature of all α-HSPs is the presence of α-crystallin domain which is the conserved sequence of about 80 amino acids[52]. α-Crystallin is known to be ubiquitous protein and it exists in many lower organisms also. From the amino acid alignment of bacterial and archaeal α-crystallin, it is observed that the α-crystallin domain is almost identical, although there is difference in N-terminal and C-terminal region.

2.2. Secondary and tertiary structure

The secondary and tertiary structures of α-crystallin is not well known due to its large size and non-availability of crystallographic or high resolution data. Circular dichroism measurements showed that α-crystallin has predominantly β-sheet structure with very little α-helices[53–56]. Early studies showed that calf lens contained 49% β-sheet and 3% helix[57]. Later estimates by Fourier-Transform Infra-Red (FT-IR) spectroscopy largely supported the above data indicating mostly β-sheet (40–50%) with some α-helical (5–10%) structure[57]. Since α-crystallin is a mixture of αA- and αB-crystallin, FTIR study was also done with recombinant αA- and αB-crystallin. αA-Crystallin was found to have 43% β-sheet and 18% α-helix compared to 48% β-sheet and 12% α-helix for αB-crystallin[58]. It was found that α-, β-, γ-crystallin are major β-sheet protein with small but varying amount of α-helix content[59].

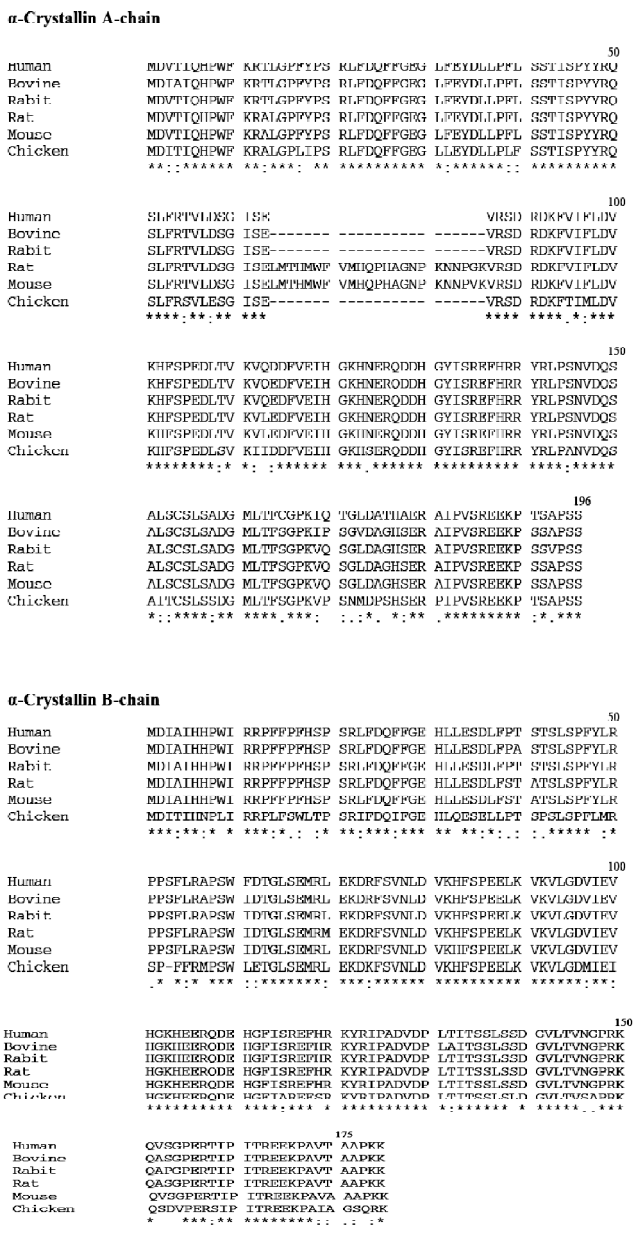

Fig. 1. Primary amino acid sequence of αA- and αB-crystallin.

Our knowledge about the tertiary structure of α-crystallin is still limited. Early investigation about the tertiary structure was done by using hydrophobic probes to find out the location of hydrophobic sites[60]. Absorption, fluorescence and near UV-CD spectroscopy provided information about the tertiary structure of lens α-, β- and γ-crystallin[61–64]. It was established that α-crystallin has both buried and exposed tryptophan residues. It was reported that each monomer of α-crystallin has a distinct N-terminal hydrophobic and C-terminal hydrophilic domain[65–67].

2.3. Quaternary structure

There are lots of controversies about the quaternary structure of α-crystallin. α-Crystallin exists in solution as high molecular mass aggregate with molecular weight ranging from 300,000 to over 1 million and it is polydisperse in nature. To describe the quaternary structure of α-crystallin, mainly three models were proposed – the three-layer models, the micelle-like models and the models of tetrameric building blocks[6,65]. Other models such as rhombododecahedral structure, a two-layer structure composed of annuli of peptides, pitted-flexiball model have also been proposed[68–70]. Crystal structure of α-crystallin is not obtained due to its polydisperse nature. By the study of cryo-electron microscopy, αB-crystallin was found to have a variable quaternary structure with a central cavity[45]. Electron microscopic study showed that α-crystallin consists of globular particles of diameter 14–18 nm and it has a torus-like structure[71,72].

3. Stability of α-crystallin

Since the eye lens does not have significant protein turnover, it does not have any valid mechanism to dispose off damaged proteins from the lens[7,28]. Since the protein must survive for the life time of a person, the crystallins, mainly α-crystallin is thought to be extremely stable protein. This notion was supported by early work which reported that α-crystallin can survive near boiling condition[73]. These conclusions have however been challenged by a number of studies which showed that α-crystallin do unfold completely at temperatures above 62°C[58,74–77]. Apart from the thermal stability studies, a number of studies focused on the denaturation patterns of α-crystallin by various ionic and non-ionic chaotropic agents, detergents etc.[66,78–81]. It has been reported that denaturation and subsequent renaturation of α-crystallin via thermal or chemical denaturation resulted in different renaturation species depending on the temperature or concentration of the chemical denaturant[82,83]. Some of the renatured α-crystallin species were functionally found to be incapable of protecting substrate protein from heat induced aggregation. For maintaining the transparency of the eye lens, high stability of α-crystallin is necessary in absence of protein turnover. A detailed understanding of the stability of α-crystallin is required to better understand the cataract problem.

4. Molecular chaperone function of α-crystallin

In 1992, Horwitz reported that α-crystallin has molecular chaperone-like properties. It prevents the heat-induced aggregation of several proteins including β- and γ-crystallin. Later, it was shown that α-crystallin also prevents disulphide bond cleavage and UV-light exposure-induced aggregation of proteins. This is a physiological function of α-crystallin, which plays vital role in maintaining the transparency of the lens *in vivo*. The functional models of chaperone function of α-crystallin have been suggested[14,69,84,85].

When proteins in the lens are damaged due to various reasons such as oxidative stress, ultraviolet radiation or any other kind of stress, they may aggregate forming large insoluble particles. α-Crystallin binds these denatured proteins and maintains the transparency of the lens. The aggregation of insulin occurs due to cleavage of disulphide bond in presence of DTT. In this case, insulin B-chain gets aggregated and α-crystallin prevents this aggregation indicating that insulin B-chain is bound by α-crystallin. α-Crystallin does not bind any substrate in its native state, it binds substrate when it tends to aggregate. α-Crystallin prevents the heat induced aggregation of many enzymes such as alcohol dehydrogenase, carbonic anhydrase, citrate synthase, lactate dehydrogenase, aldolase, α-glucosidase etc. The aggregation of the natural substrates of α-crystallin, such as β-crystallin, γ-crystallin, aldose reductase etc. is also prevented by α-crystallin[28,30–32 34,86–89]. The heat-induced aggregation of recombinant human aldose reductase was suppressed by recombinant human αA-crystallin[87]. When wild type (WT) αA-crystallin was added to aldose reductase solutions, apparently complete suppression was observed by the addition of approximately stoichiometric amounts of αA subunits relative to aldose reductase (Fig. 2A). The protection of aggregation in presence of the W9F mutant showed that there was no alteration of chaperone-like activity (Fig. 2B). On the other hand, only partial suppression of aggregation was observed in presence of R157STOP mutant, even with addition of an almost 2-fold molar excess of the truncated subunits (Fig. 2C). αA-Crystallin and its mutants in different concentrations were taken to assess the chaperone activity.

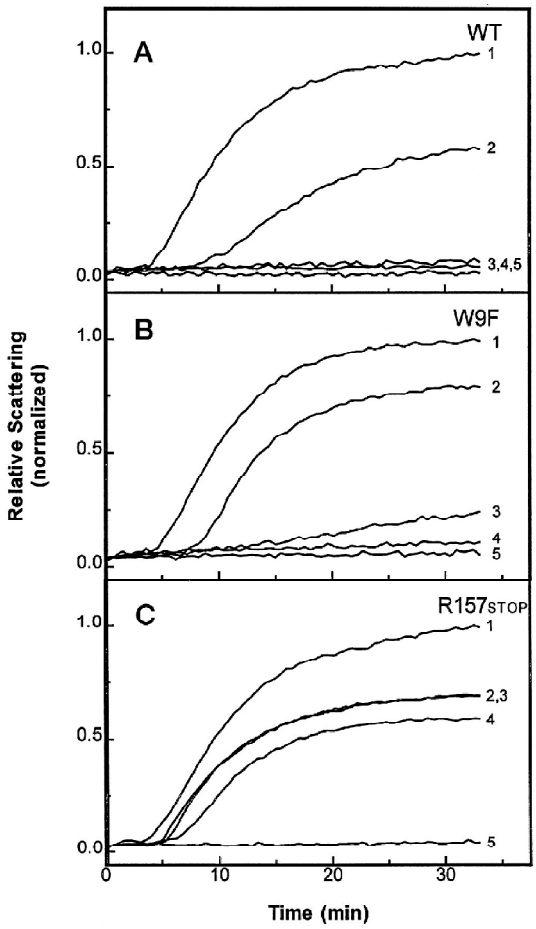

Fig. 2. Aggregation of human aldose reductase in presence of αA-crystallin and its mutants. Aggregation curves of aldose reductase (0.6 μM, 22 μg/ml) in presence of αA-crystallins were obtained by measuring A_{360} of protein solutions of total volume of 0.5 ml. In all cases, absorbance of samples containing aldose reductase alone (curves 1) and α-crystallin subunits alone (~1 μM, curves 5) was measured. **A:** aggregation in presence of WT αA-crystallin incorporated to a final concentration of 5, 10 and 20 μg/ml (curves 2–4, respectively) corresponding to 0.25, 0.5 and 1 μM. **B:** aggregation in presence of the W9F αA mutant incorporated to a final concentration of 5, 10 and 20 μg/ml (curves 2–4, respectively) corresponding to 0.25, 0.5 and 1 μM. **C:** aggregation presence of R157STOP αA mutant incorporated to a final concentration of 5, 10 and 20 μg/ml (curves 2–4, respectively) corresponding to 0.28, 0.56 and 1.11 μM (Reproduced with permission from *J. Biol. Chem.*, 1996, **271**, 31973. © the American Society for Biochemistry and Molecular Biology. Ref. 87).

Proteins contain both hydrophilic and hydrophobic side chain residues. Individual protein has its own folding pattern in which they arrange hydrophobic residues in such a way that some hydrophobic residues may be buried in the interior and others may be exposed at the surface. The several properties of protein like conformation, solubility, ligand binding, aggregation etc. are largely determined by hydrophobic interactions. Relationship between the hydrophobicity and

physico-chemical properties of proteins has been the subject of interest[90–92]. Several experiments have been done for determination of hydrophobicity of protein. One of these methods is fluorescent probe method[93]. α-Crystallin is known to have significant amount of exposed surface hydrophobic sites that can bind to various hydrophobic probes such as ANS and bis-ANS[27,40,60,82,83]. The exposed hydrophobic sites were also thought to be involved in the binding of target substrate proteins to α-crystallin during its chaperone activity[30,58 74,94,95]. Several workers have shown a direct correlation between chaperone activity and exposed hydrophobicity of α-crystallin[27,33,75,96].

The chaperone activity of α-crystallin was enhanced with increase of temperature[27,58,97,98]. The conformational change of α-crystallin due to heating lacked reversibility[27]. It was seen that in presence of 3 *M* urea, quaternary structure of α-crystallin was perturbed and its chaperone activity against photoaggregation of γ-crystallin was enhanced[30]. Thus not only the thermotropic changes, quaternary structural perturbation of α-crystallin by nonthermal mode also results in enhancement of chaperone activity. It was seen that thermal assay required less α-crystallin for protection than non-thermal assay[28,99]. The mechanism of chaperone activity is still unknown.

There were lots of work to know the substrate binding sites of α-crystallin. The chaperone activity of α-crystallin was reduced after binding with bis-ANS[58,74,82,83,100–105]. As the glycation of α-crystallin reduces its chaperone activity, to determine whether it is due to an alteration of the hydrophobic chaperone site in α-crystallin, the interaction of glycated α-crystallin with hydrophobic probe ANS was measured[83]. Glycation of α-crystallin was carried out in 0.1 *M* phosphate buffer, pH 7.0, using 10 mg/ml protein and 20 mM L-ascorbic acid. After incubation at 37°C for 4 weeks, the reaction mixture was dialyzed and the glycated protein was treated with β_L-crystallin for chaperone activity. The interaction of glycated α-crystallin (0.25 μM) with ANS was examined by fluorescence measurement taking excitation wave length at 390 nm and the emission wave length at 490 nm. The ratio of protein to probe was approximately 1:50. α-Crystallin incubated without ascorbic acid and processed similarly was used as the control. Glycation of α-crystallin decreased ANS binding. Effect of glycation on chaperone activity of α-crystallin was shown taking β_L-crystallin as substrate. The chaperone activity of α-crystallin was reduced for glycated protein (Fig. 3). This study suggests that the glycation-induced loss of chaperone activity may be due to the common binding site of the hydrophobic probes and substrate proteins to the α-crystallin. There were other reports that indicate hydrophobic probes and substrate proteins have common binding site[106,107].

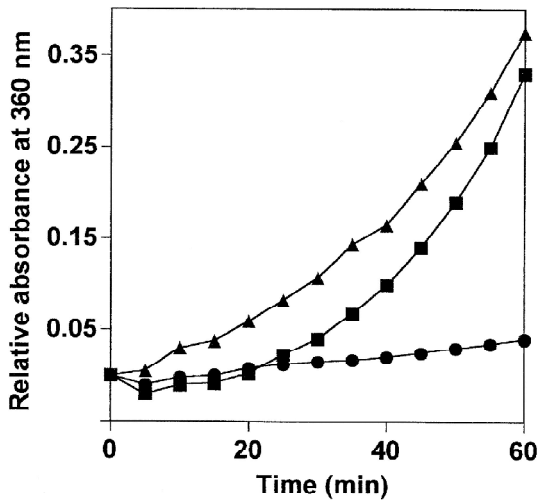

Fig. 3. Effect of glycation on chaperone activity of α-crystallin. 200 μg of β_L-crystallin and 30 μg of α-crystallin or glycated α-crystallin were used in this study. **squares:** β_L-crystallin, **circles:** β_L-crystallin + α-crystallin, **triangles**: β_L-crystallin + glycated α-crystallin (Reproduced with permission from *J. Biol. Chem.*, 1998, **273**, 15474. © the American Society for Biochemistry and Molecular Biology. Ref. 83).

It was observed that bis-ANS was bound to the N-terminal region of αB-crystallin in rat[105] and also to the N-terminal region of bovine αA-crystallin[103]. It reveals that N-terminal region of α-crystallin contributes to substrate binding. α-Crystallin domain has also contribution to the substrate binding[83,107]. Cross-linking studies with denatured alcohol dehydrogenase (ADH) to bovine αA- and αB-crystallin showed that two binding sites were located between residues 57 and 69 and residues 93 and 107[106,107]. The same studies with denatured mellitin, a 2.8 kDa hydrophobic polypeptide, showed the single binding site between residues 75 and 82[106,107]. A synthetic 19-residue peptide from the α-crystallin domain (residues 70–88) of human αA-crystallin prevented thermal aggregation of ADH[107]. Thus multiple regions are involved in substrate binding. The thiol-containing region of αA-crystallin (at positions 131 and 141) is involved in chap-

erone activity. Thiol residues may serve as markers to probe possible substrate binding sites or substrate induced changes in this region[108].

5. Oligomeric structure and chaperone activity

It is known that all sHSPs including α-crystallin are molecular chaperones[6,29]. Formation of large oligomeric complexes is the hallmark of sHSPs. There was a notion that large oligomeric structure of α-crystallin or other sHSPs is responsible for their chaperone-like activity[10,14]. The reason of this belief is that both naturally occurring α-crystallin and mutated variants, that do not form oligomers, are poor chaperones[109–111]. It has been reported that for bacterial α-heat shock proteins, the chaperone activity was strongly coupled to multimerization[112–114]. Cleavage of any region of the sequence from *B. japonicum* HSP proteins that reduced oligomerization also reduced chaperone activity[114]. A monomeric form of HSP 12.6 from *Caenorhabditis elegans* had no chaperone activity[110]. Tetrameric sHSP members of the same family were devoid of the chaperone activity[109]. Again HSP 20 from rat assembled into a dimer and had very little chaperone activity[111]. Although lenticular α-crystallins from higher organisms have many properties different from that of lower organisms, correlation between oligomerization and chaperone activity has been generally assumed. All these data indicate that there is a great deal of controversy regarding the relationship between the oligomeric size and chaperone activity of α-crystallin.

6. Cataract

The lens is a unique organ. It continues to grow throughout life yet lacks a blood supply and exhibits no protein turnover. The crystallin proteins in its centre are as old as the individual. To maintain lens transparency and the high refractive index necessary for vision, the crystallins must remain stable. With age, extensive modification occurs to the crystallins, i.e. mutation, deamidation, racemisation, phosphorylation, truncation, glycation etc. which collectively affect their structure, solubility and potentially lead to precipitation, lens opacification and finally cataract formation. Cataract can have many causes.

6.1. Causes of cataract

Mutation: Various mutations in the α-crystallin genes have been indicated to cause cataract diseases in human. Mutation in one of the lenticular proteins can cause cataract[115–118]. Mutation (R116C) that generated high polydispersity and increased membrane affinity of αA-crystallin, showed lower chaperone activity and caused congenital cataract[115,117]. The αA R49C mutant protein showed autosomal dominant cataract[118]. The mutation V124E in mouse αA-crystallin also caused dominant cataract[116].

Chaperone activity of both wild-type and R12C mutant αB-crystallin was compared[119]. Both wild-type and R12C mutant αB-crystallin were subjected to thermal stress and also treated with calcium. The thermal and calcium-induced aggregation was significantly prevented by mutant αB-crystallin compared to wild type αB-crystallin. In the chemically induced aggregation system, aggregation assay was performed at 37°C taking insulin (0.3 mg/ml) as the target protein and chaperone activity of these proteins was also performed in heat-induced aggregation system using γ-crystallin (0.3 mg/ml) at 60°C (Fig. 4). Structural analysis and chaperone activity assessment of wild-type and R12C αB-crystallin was also performed in presence of calcium ion (Fig. 5). The recombinant αB-crystallin was incubated with different concentrations of calcium ions for one week at 37°C. At the end of the incubation, the protein samples were diluted and used for structural investigation by fluorescence spectroscopy. Tryptophan fluorescence spectra were taken with protein samples of concentration 0.15 mg/ml (Fig. 5a). Protein samples (0.15 mg/ml) were incubated with ANS (100 μM) for 30 min and then emission spectra were recorded (Fig. 5b). Chaperone activity assay was performed in presence of 5 mM calcium using insulin (0.3 mg/ml) as the target protein (Fig. 5c). Chaperone activity was shown in terms of percentage of protection (Fig. 5d). This study indicates that chaperone activity of αB-crystallin is slightly enhanced due to mutation and also explains the non-cataractogenic nature of R12C mutation in αB-crystallin. The same mutation was done in αA-crystallin and in presence of calcium, both wild-type and mutant R12C αA-crystallin showed reduced chaperone activity[120]. The mutant R12C αA-crystallin showed significantly higher reduction of chaperone activity than wild-type protein. It was suggested in presence of calcium and due to mutation, some conformational changes occur in αA-crystallin and it may play an important role in the pathomechanism of the cataract development.

A highly reactive oxygen and nitrogen species, peroxynitrite (PON), which is produced in eye, has significant contributions to a variety of ocular disorders. The struc-

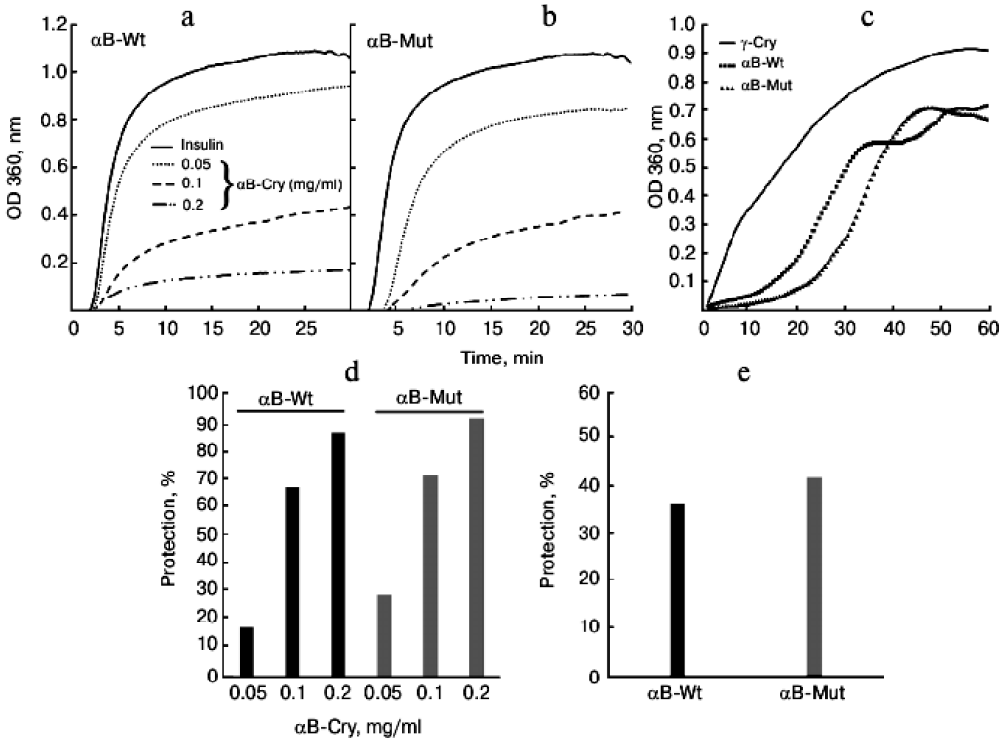

Fig. 4. Comparison of chaperone activity of wild-type and R12C mutant αB-crystallin. Insulin (0.3 mg/ml) was used as target protein and aggregation was induced in presence of 20 mM DTT at 37°C. **a, b:** Chaperone activity of wild-type and mutant αB-crystallin. **c:** chaperone activity of these proteins when γ-crystallin (0.3 mg/ml) was used as target protein at 60°C. These experiments were done in 50 mM Tris buffer, pH 7.2, containing 100 mM NaCl. **d, e:** The percentages of protection obtained by these chaperones in the DTT-induced aggregation of insulin and in the thermally induced aggregation of γ-crystallin, respectively (Reproduced with permission from *Biochemistry (Moscow)*, 2016, **81**, 122. © Pleiades Publishing Ltd. Ref. 119).

tural characteristics, chaperone-like activity and conformational stability of R54C mutant αA-crystallin were studied upon modification with PON and in presence of three antioxidant compounds such as ascorbic acid (ASA), glutathione (GSH) and N-acetylcysteine (NAC). The chaperone-like activity of R54C mutant αA-crystallin was enhanced against aggregation of γ-crystallin and insulin upon modification with PON. In addition, different antioxidant compounds took a major role in neutralizing the PON damaging effects on structural integrity and stability of this protein. This study suggests that for protection of lens crystallins against PON-mediated structural damages and cataract development, antioxidant-rich foods or potent antioxidant can be used[121].

The solubility and stability against thermal and guanidine hydrochloride-induced denaturation of βB2-crystallin, the fraction of eye lens protein, was significantly decreased by two mutations, W59C and W151C. In presence of UV light, these two congenital cataract-causing mutated proteins form aggregates. It was suggested that the conserved Tryptophan residues might play a more important role in the correct folding and structural integrity of β-crystallin domains than in γ-crystallins[122]. Congenital cataract is the leading cause of childhood blindness and progressive neuro degeneration of the optic nerve in glaucoma. A Triple Mutation of βB2-crystallin leads to form aggregates and develops cataract and glaucoma. It is associated with mislocalization to the mitochondria along with decreased mitochondrial function in retinal neurons and lens epithelial cells[123].

The P94S mutation gives rise to a dominant lamellar cataract. L69P is associated with microphthalmia with cataract[124]. G64W is associated with congenital cataract and microcornea[125]. The insertion of cytosine at position 1195 of CX46 cDNA is a novel mutation site that is associated with the hereditary dominant cataracts in a Chinese family[126]. Several destabilizing mutations in crystallin genes are linked with human autosomal dominant hereditary cataracts. The α-crys-

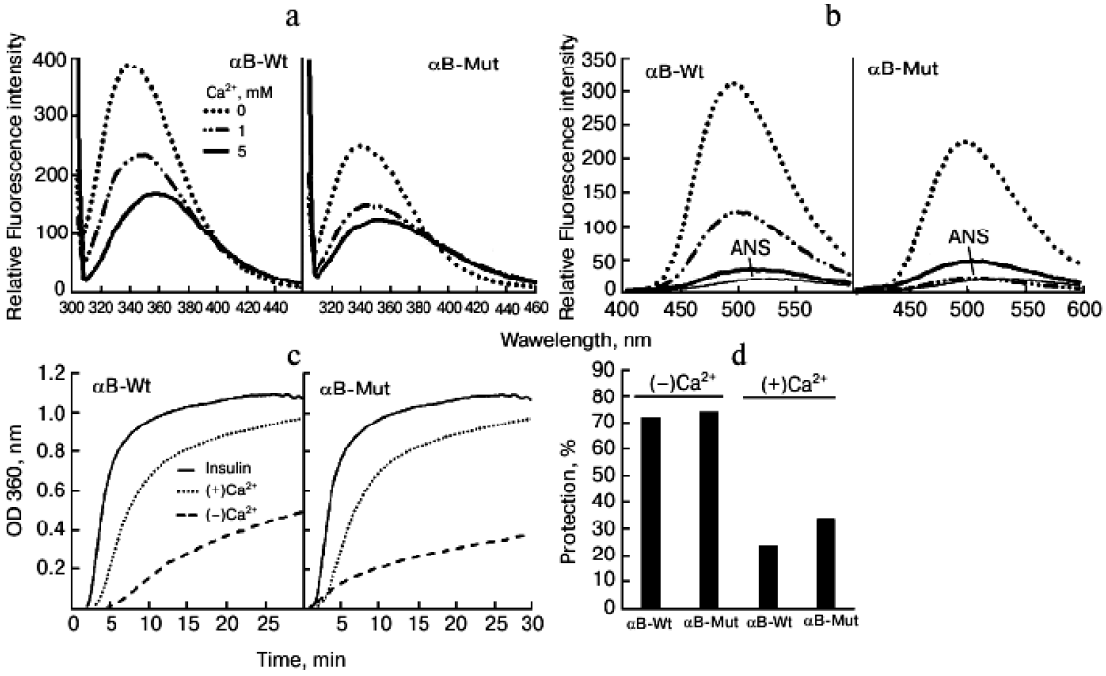

Fig. 5. Structural analysis and chaperone activity of wild-type and R12C αB-crystallin in presence of calcium ion. The recombinant αB-crystallin was incubated with different concentrations of calcium ions (0, 1 and 5 mM) for one week at 37°C and the protein samples were diluted in 50 mM Tris buffer, pH 7.2, containing 100 mM NaCl at 25°C. **a:** Tryptophan fluorescence spectra with protein samples (0.15 mg/ml), where excitation wave length was 295 nm and emission wave length range was 300–500 nm. **b:** Protein samples (0.15 mg/ml) were incubated with ANS (100 μM) for 30 min and then spectra were recorded taking excitation wave length at 365 nm and emission wave length in the range 400–600 nm. **c:** Chaperone activity was assessed in 50 mM Tris buffer, pH 7.2, containing 100 mM NaCl in presence of 5 mM calcium. Insulin (0.3 mg/ml) was used as the target protein in presence of 20 mM DTT. **d:** Chaperone activity is expressed in terms of percentage of protection (Reproduced with permission from *Biochemistry (Moscow)*, 2016, **81**, 122. © Pleiades Publishing Ltd. Ref. 119).

tallin mutations αA-R49C and αB-R120G are associated with autosomal dominant human cataracts[127].

Deamidation: Aging of the lens of the eye is accompanied by extensive deamidation of the lens specific proteins named crystallins. Deamidation of proteins is one of the most prevalent post-translational modifications found upon aging, and in age-onset diseases. Specific asparagine and glutamine residues are often selectively deamidated during this process. Deamidated crystallins are increased in the insoluble proteins and may contribute to cataracts.

The effect of deamidation on stability, formation of intermediates and aggregation of βB2-crystallin was studied by the techniques such as dynamic light scattering, differential scanning calorimetry and small angle X-ray scattering. At the interface of the βB2-dimer, unfolding and aggregation occurred on heating due to deamidation. Deamidation had a greater effect at Q70 in the N-terminal domain than at Q162 in the homologous C-terminal domain. It was observed that the native α-crystallin was only able to partially rescue this aggregate[128]. The mechanism of deamidation altering interactions between αA-crystallin and βB2-crystallin was investigated by detecting changes in solvent accessibility upon complex formation during heating by using high-resolution mass spectrometry. It was observed that deamidation did not disrupt specific αA/βB2 interactions but favored aggregation before complex formation with αA-crystallin. This study suggests that deamidation contributes to cataract formation through destabilization of crystallins before they can be rescued by α-crystallin[129].

Modification of Asn and Asp over time may contribute to denaturation of proteins in the human lens. An accelerated rate of deamidation or racemization at selected sites of γS-crystallin may contribute to cataract formation[130]. It was reported that the secondary structure of the protein remained intact, but minor changes occurred in the tertiary structure due to deamidation of specific asparagine and glutamine

residues in human recombinant γS-crystallin. The results indicate that there was no significant change in either protein structure or stability[131]. Deamidation of N76 in human γS-crystallin promotes dimer formation[132]. The effect of Glu147 deamidation on chaperone activity of αA-crystallin was studied for a variety of aggregating proteins and it was suggested that deamidation of Glu147 in human αA-crystallin is common in aged cataractous lenses[133]. Deamidated Q147E αA-crystallin was structurally characterized using various techniques including NMR, circular dichroism and fluorescence spectroscopy and dynamic light scattering. Q147E αA-crystallin showed slightly reduced chaperone activity than wild-type protein. As αA-crystallin is the major lens protein, even a small loss of function could combine with other sources of age-related damage to the crystallins and play a crucial role in cataract formation[134].

Other causes: There are other causes of cataract which can be discussed briefly. It can be one of the symptoms of systemic disease, for example, diabetes is a risk factor for cataract[135–137]. Aging can also contribute to cataract. It was reported that during aging, α-crystallin formed high molecular weight complexes with other proteins in lens[138–142]. It was also reported that membrane-associated high molecular weight α-crystallin complexes showed significantly reduced chaperone-like activity and formed cataract[143]. Phospholipid vesicles were used as the primary binding template for measurement of chaperone-like activity of membrane-associated complexes because Dipalmitoylphosphatidylcholine (DPPC) and sphingomyelin (SPH) vesicles suspensions are essentially clear while fractionated lens membranes cause significant light scattering. Two different mixtures for each α-crystallin homocomplex were taken and the chaperone-like activity was measured. First, DPPC vesicles were incubated with and without α-crystallin homocomplexes for 15 h at 37°C. As controls, α-crystallin homocomplexes were incubated without vesicles under identical conditions. Then the sample containing a mixture of both bound and unbound α-crystallin was used in heat-induced chaperone assay with human aldose reductase (HAR) as the substrate. For wild-type (WT) αA-crystallin, a molar ratio of 1:0.5 (HAR to WT αA) was taken, whereas the ratio was 1:1 for WT αB-crystallin. A small difference in the chaperone-like activity was observed for WT αA mixtures as compared to the vesicle-free α-crystallin controls, although the total amount of HAR aggregation remained similar. For WT αB, the mixture containing both bound and unbound protein had indistinguishable chaperone-like activity compared to the soluble protein alone. Crystallin-free reactions confirmed that the presence of vesicles did not alter the amount of HAR aggregation, while α-crystallin-only controls showed no aggregation in absence of HAR (Fig. 6). The aggregation and cross-linking of crystallin fragments lead to cataract development. Several post-translational modifications of crystallin fragments form aggregates with water soluble high mo-

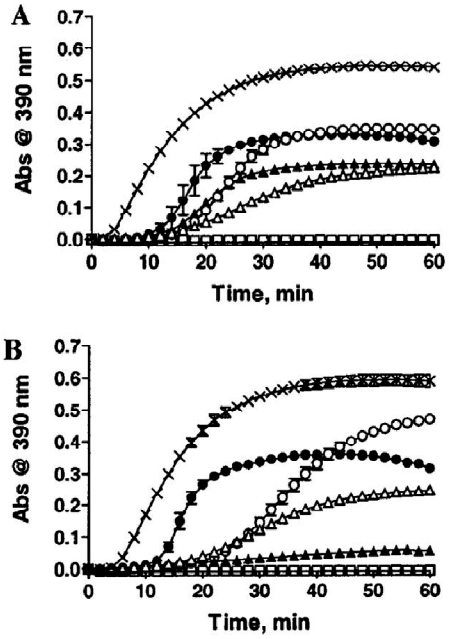

Fig. 6. Chaperone-like activity of membrane-bound α-crystallin. **(A)** WT αA-crystallin (1 nmol) and WT αB-crystallin (2 nmol) were incubated with DPPC vesicles, the mixture of bound and soluble α-crystallin was assayed by heat denaturation with HAR (2 nmol). A small difference was observed between WT αA in presence (Δ) and absence ( ) of DPPC vesicles. There was no appreciable difference in the curves with WT αB in presence (o) and absence (•) of DPPC vesicles. For an aggregation positive control, HAR was incubated with DPPC vesicles but there was no α-crystallin (×). For an aggregation negative control, α-crystallin was incubated with DPPC vesicles but there was no HAR (□). **(B)** WT αA and WT αB were bound to SPH vesicles and the purified vesicles with α-crystallin bound (2 nmol of α-crystallin/assay) were used in assays as before (with 2 nmol of HAR) with similar aggregation positive (×) and negative (□) controls. A considerable difference was noted between SPH-bound WT αA (Δ) and unbound WT αA ( ). SPH-bound WT αB (o) and unbound WT αB (•) showed a significant reduction in activity (Reproduced with permission from *Biochemistry*, 2002, **41**, 483. © American Chemical Society. Ref. 143).

lecular weight proteins and play a major role in development of human cataract[144].

6.2. Prevention of cataract

Cataract is still one of the major causes of adult blindness. Extensive research is ongoing to prevent blindness. Currently the only available treatment for cataract is surgery, surgical replacement of cataractous lens, which can not be the acceptable solution in developing countries. In selenite-induced cataract, it was suggested that hesperetin can prevent the decreasing lens chaperone activity and α-crystallin water solubility by administered of selenite[145]. It was reported that the differential loss of α-crystallin in the human lens epithelium could be associated with the different mechanisms of cataractogenesis in age-related versus congenital cataracts, subsequently resulting in different clinical presentations[146]. To investigate the gene expression of αA- and αB-crystallin in the lens epithelium of age-related and congenital cataracts, the mRNA expression levels were detected by real-time PCR assays. Total RNA was extracted from the human lens epithelium specimens. The mRNA levels of αA- and αB-crystallin were significantly reduced in case of age-related and congenital cataracts. For αA-crystallin in the age-related cataract group the gene expression was approximately 0.55 fold than that of the normal control, whereas αA-crystallin gene expression level in the congenital cataract group was 0.25 fold than that of the normal control group (Fig. 7A). On the other hand, the gene expression level in the age-related cataract group was approximately 0.8 fold than that of the normal control, but gene expression level in the congenital cataract group was only 0.65 fold than that of the normal control in case of αB-crystallin (Fig. 7B). The reduction of αA-crystallin gene expression in the congenital cataract group was approximately 1.67 times greater than that of the age-related cataract group (Fig. 7A), whereas the reduction of αB-crystallin gene expression was about 1.75 times greater in the congenital cataract group than in the age-related cataract group (Fig. 7B). These results indicated that the reduction of both αA- and αB-crystallin transcripts differed dramatically in the lens epithelium of the age-related versus congenital cataracts. The two gene expression levels were more significantly reduced in the congenital cataract group.

Based on the gene expression changes of αA- and αB-crystallin in age-related and congenital cataract lens epithelium, the soluble protein levels of αA- and αB-crystallin were assessed by western blots to know whether a similar trend could be found for the protein levels. It was observed that the protein levels of both soluble αA- and αB-crystallin were significantly reduced in age-related and congenital cataracts

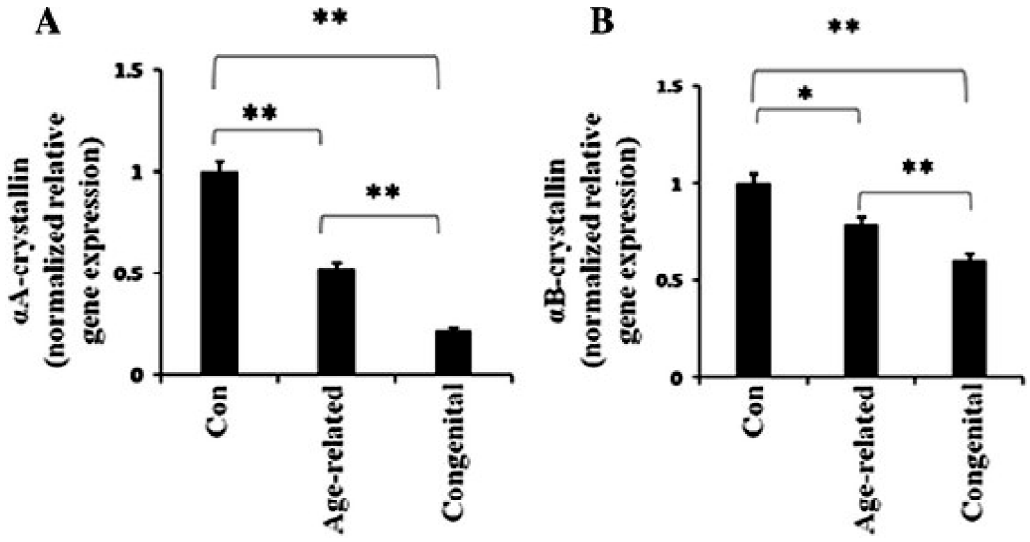

Fig. 7. αA- and αB-crystallin relative gene expressions in age-related and congenital cataract lens epithelium. RNA was extracted from human lens capsule epithelium specimens. Real-time PCR was performed for detecting the RNA levels of αA-crystallin (**A**) and αB-crystallin (**B**) in each group. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the internal control gene (mean ± SD, $n = 3$). *$P < 0.05$, **$P < 0.001$ (Reproduced with permission from *BMC Ophthalmology*, 2016, **16**, 67. © Springer Nature. Ref. 146).

when compared with the normal control group. Soluble αA-crystallin in the age-related cataract was approximately 0.75 fold than that of the normal control group and in the congenital cataract it was approximately 0.4 fold than that of the normal control group (Fig. 8A). In age-related cataract, the protein levels of soluble αB-crystallin were approximately 0.65 fold than that of the normal control group, and in the congenital cataract group, the levels were approximately 0.45 fold than that of the normal control group (Fig. 8B). For soluble αA-crystallin, the protein reduction level in the congenital cataract group was approximately 2.4 fold greater than that of the age-related cataract group. But for αB-crystallin, that was approximately 1.57 fold greater than that of the age-related cataract group. These results indicate that the reduction was more severe in the congenital cataract and the reduction level of soluble α-crystallin in the lens epithelium might be one of the contributing factors that lead to the different appearances of age-related versus congenital cataract. More comprehensive study is required to know the underlying relationships between α-crystallin expression and cataract formation.

The classical drug therapies for ocular diseases are either systemic or topical. In both, the delivery and bioavailability of the drug are limited because of the presence of numerous barriers that isolate the eye from the external environment. The normal corneal layer has several tight barriers, hydrophobic and hydrophilic. These layers generally prevent water-soluble molecules and hydrophobic microparticles from entering the eye through cornea due to their size. Nanoparticles could easily penetrate the eye through the cornea since these particles are very small and also hydrophobic. A new type of ophthalmic treatment used nowadays is nano eye-drops which have increased strength and reduced side effects. A commercially available glaucoma treatment drug, brinzolamide formulated as micro-sized structures is used in nano eye-drops. Fabrication of the nanoparticles of brinzolamide increases the eye penetration rate and results in high drug efficiency compared to commercially available brinzolamide eye-drops. Also, the nano eye-drops are non-toxic to the corneal epithelium after repeated administration for 1 week[147].

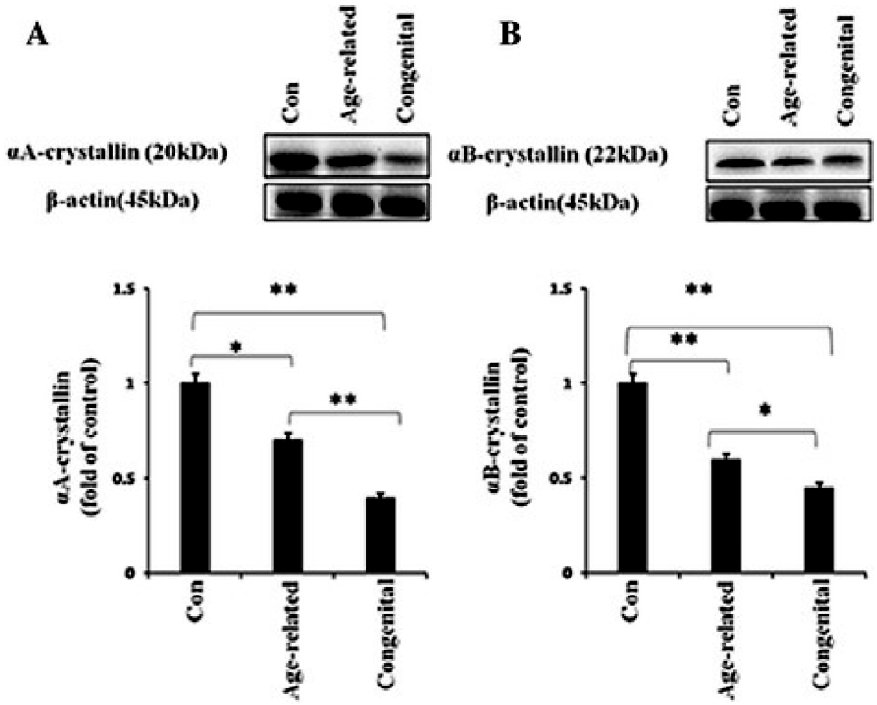

Fig. 8. Soluble αA- and αB-crystallin protein expressions in age-related and congenital cataract lens epithelium. Proteins were extracted from human lens capsule epithelium specimens. Western blot assay was performed for detecting the αA-crystallin (**A**) and αB-crystallin (**B**) protein levels of each group. β-actin was used as the internal control. (mean ± SD, $n = 3$). *$P < 0.05$, **$P < 0.001$ (Reproduced with permission from *BMC Ophthalmology*, 2016, **16**, 67. © Springer Nature. Ref. 146).

The leading cause of cataract formation is glycation. An alternative non-surgical approach for cataract can be the use of a suitable chemical or biochemical agent that would either prevent glycation of lens proteins or reverse the formation of cataract. Glycation is a nonenzymatic interaction between glucose and protein. The glycated protein may then react with any other protein resulting cross-linking product. Cataract is very much found in diabetic patients because of high amount of sugar in their blood. Curcumin conjugated gold nanoparticles show anti-cataract activity but further studies are required to ensure its validity[148]. N-Acetyl-carnosine is very well-known agent that reverses cataract formation, although there is some reservation regarding the effectiveness of N-acetyl-carnosine eye drops[149].

Concluding remarks

Cataract is the leading cause of vision loss worldwide. The development of nonsurgical treatments is very much essential for preventing or reversing cataract. The nanotechnology offers a new settlement for ophthalmological treatments and it plays a considerable role in the development of biosensors, diagnostic labelling strategies, detection monitors in ophthalmology. The nanotechnology offers the production of nanoparticles. Thus it can provide most suitable solutions for drug delivery to the eye. The nano eyedrops may have applications as a next generation ophthalmic treatment. The prevention of glycation in presence of curcumin nanoparticles could lead us to a possible treatment procedure for cataract in future. Nanotechnology offers the therapeutic solutions required to avoid physical barriers and to increase bioavailability as well as persistent and controlled delivery. None of the drug formulations will be approved for use unless efficient delivery is promised.

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