Modulation of α-crystallin chaperone activity: A target to prevent or delay cataract?

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Critical Review

Modulation of α-Crystallin Chaperone Activity: A Target to Prevent or Delay Cataract?

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Summary

Cataract, loss of eye lens transparency, is the leading cause of blindness worldwide. α-Crystallin, initially known as one of the major structural proteins of the eye lens, is composed of two homologous subunits αA- and αB-crystallins. It is convincingly established now that α-crystallin functions like a chaperone and plays a decisive role in the maintenance of eye lens transparency. The functional ability of α-crystallin subunits is to act in cooperation as molecular chaperones to prevent the cellular aggregation and/or inactivation of client proteins under variety of stress conditions. However, chaperone-like activity of α-crystallin could be deteriorated or lost during aging or under certain clinical conditions because of various genetic and environmental factors. This review will focus specifically on relevance of α-crystallin chaperone function to lens transparency. In particular, we reviewed the studies that demonstrate the modulation of α-crystallin chaperone-like activity and discussed the possibility of chaperone-like activity of α-crystallin as a potential target to prevent or delay the cataractogenesis. © 2009 IUBMB

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Keywords α-crystallin; chaperone-like activity; modulation of chaperone function; prevention of cataract.

INTRODUCTION

Vision is the most precious of all the senses and is achieved by a set of ocular tissues working in concert. The transparent eye lens functions to focus the light entering the cornea onto the retina without any spherical aberration. Lens transparency is maintained by a liquid-like, short-range order of highly concentrated solutions of crystallin proteins (*1*). Crystallins are lenticular structural proteins and in mammals they are comprised of three families: α-, *β*- and *γ*-, each with distinct subunits. Furthermore, necessary refractive index in the lens is achieved by the absence of cellular organelles in the postmitotic lens fiber cells. Cataract is defined as opacity of the transparent eye lens, which interferes with transmission of light onto the retina and results in the vision impairment. Cataract is a leading cause of blindness worldwide and is responsible for ∼40–80% of the estimated 45 million cases of blindness that occur across the globe (*2*). Two possible mechanisms, which are not mutually exclusive, are proposed to cause the cataract. The first is a condensation phenomenon, whereby lenticular opacity results from loss of solubility and an increase in the nucleation rate of the crystallins (*3*). The second mechanism explains cataract as a conformational disorder (*4*), where unfolding or destabilization of the lens crystallins lead to cataractogenesis. Nevertheless, the occurrence of cataract, which arises from aggregation and precipitation of crystallins, reveals that there are shortcomings with respect to lens protein stability. During cataractogenesis, formation of high molecular mass (HMM) aggregates cause fluctuations in protein density, resulting in light scattering and a concomitant decrease in transparency. Despite the advanced understanding about the pathogenesis of cataract, the only treatment available so far is surgical extraction of the lens and replacement with an intraocular lens. Although surgery offers excellent outcomes for cataract, besides possible postsurgical complications, an artificial lens does not have the overall optical qualities of a normal lens. Furthermore, good surgical outcomes and access to care are limited for people of lower socioeconomic status in parts of the developing world, where 90% of cataract blindness occurs. Hence, biochemical solutions or pharmacological interventions that can maintain the transparency of the lens are very much required. Recognition of chaperone-like function of α-crystallin has opened the new avenues for the treatment of cataract and other insoluble protein disorders. By now, it is fairly acknowledged that chaperoning ability of α-crystallin is instrumental in the maintenance of eye lens transparency, and

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decreased chaperone-like activity of α-crystallin is associated with various types of cataract. Therefore, a better understanding of safeguarding the α-crystallin chaperone activity may aid the development of therapeutic strategies that could evade the need for surgery. This review summarizes the significance of modulation of α-crystallin chaperone activity in consequence to prevent or delay the cataract.

α-CRYSTALLIN

α-Crystallin, one of the three major lens crystallins, is a representative member of the small heat shock protein (sHsp) family (*5*). sHsp are ubiquitous proteins that are vigorously induced under stress and disease conditions (*6, 7*). In the lens, α-crystallin exists as a highly heterogeneous aggregate with sizes ranging from 300 kDa to over 1,000 kDa with an average size about 700 kDa. α-Crystallin oligomer is composed of ~40 subunits derived from two gene products αA and αB. αA is 173, whereas αB is 175 amino acids long and both share 57% sequence similarity. In most of the vertebrate lenses, αA and αB subunits exist in the ratio of 3:1, though the ratio varies with species and also with aging (*8*). Description about the structure of α-crystallin is out of scope of this review. It was first recognized that sHsp from Drosophila (sHsp27, sHsp26, sHsp23, sHsp22) and α-crystallin from vertebrate eye lens contained a homologous C-terminal domain of ~90 residues (*9*), later named as the "α-crystallin domain." Approximately a decade later, it was shown that αB-crystallin expression, similar to sHsp, was indeed induced by heat shock (*10*). Induction of αB-crystallin is also seen in other stress stimuli such as cadmium, arsinite, osmotic, or hypertonic stress and clinical conditions such as neurodegenerative diseases and diabetes (*11–14*). The αB-crystallin promoter region shows the presence of a canonical stress-inducible heat shock element (HSE), which can be induced by heat shock factors (*15*). Furthermore, like other sHsp, α-crystallin subunits are low monomeric mass proteins (~20 kDa) that form large oligomeric assemblies and show association with the nucleus under stress conditions (*16–18*). Although thought to be merely structural proteins contributing crystalline properties to the lens, αA- and αB-subunits are also expressed in a number of other ocular and nonocular tissues (*19*). Similar to other members of sHsp, αA- and αB-crystallins enhance stress resistance (*20, 21*), modulate the redox state (*22, 23*), inhibit apoptosis (*24–27*), and regulate membrane fluidity (*28*). αB-crystallin was shown to interact with several proteins involved in cellular differentiation and signaling such as FGF2, NGF-β, and VEGF as assessed by protein pin arrays (*29*) and cell organelle reorganization (*30*). αB-crystallin interacts with a number of cytoskeleton filament proteins including phakinin, filensin, desmin, glial fibrillary acidic protein, vimentin, and actin where it functions in the organization and stabilization of the filament networks formed by these proteins (*31*). The cytoskeleton undergoes extensive remodeling during morphogenesis and stress conditions, thus interaction of α-crystallin with cytoskeletal proteins represents an important cellular function of α-crystallin. A recent review described about emerging new roles for αB-crystallin in several pathologies associated with protein conformation and cancer (*32*). This study also highlights the importance and need of drugs that could modulate the activity and expression of αB-crystallin subunit.

MOLECULAR CHAPERONE-LIKE FUNCTION OF α-CRYSTALLIN AND EYE LENS TRANSPARENCY

Molecular chaperones are a functional class of unrelated families of protein that assist the correct noncovalent assembly of other polypeptide-containing structures *in vivo*, but are not components of these assembled structures when they are performing their normal biological function. The predominant function of molecular chaperones is the prevention of incorrect associations and aggregation of unfolded polypeptide chains.

For the first time, chaperone-like activity of α-crystallin was demonstrated by its ability to prevent the thermal aggregation of other lenticular proteins such as β- and γ-crystallins and nonlenticular protein alcohol dehydrogenase (*33*). It was thus proposed that apart from contributing crystalline properties to the lens, α-crystallin could serve as a molecular chaperone, protecting damaged or aged lens proteins and enzymes from aggregation that would otherwise lead to light scattering and cataract formation (*34*). Since then, numerous studies have reported the ability of α-crystallin to protect various model (client) proteins from aggregation/inactivation due to various stress conditions (Reviews, *35–38*). Most importantly, the ability of α-crystallin to confer protection to client proteins such as γ-crystallin and G6PD against UV-irradiation (*39–41*) and prevent glycation-induced inactivation of various enzymes (*42–45*) may be more relevant in the maintenance of lens transparency, because UV-irradiation and nonenzymatic glycation of lens proteins are considerable risk factors for cataractogenesis. However, unlike classical chaperones (*e.g.*, GroEL, DnaK and SecB), α-crystallin does not interact with fully extended or completely unfolded proteins. Instead, it complexes only with compact, severely compromised proteins that are about to precipitate out of the solution, that is, aggregation-prone partially folded intermediates of client proteins (*46–50*). α-Crystallin interacts with aggregation-prone nonnative proteins through surface-accessible hydrophobic surfaces and holds them in a refolding competent state (*38*).

The significance of α-crystallin in maintaining the transparency of lens was clearly evident in experiments, where the total soluble lens homogenate subjected to heat-induced aggregation at 60°C, showed a slight aggregation (*41, 51*); whereas, a part of α-crystallin removed from lens homogenate by ultracentrifugation resulted in increased aggregation of remaining lens homogenate (*51, 52*). These findings may be more relevant to *in vivo* chaperone function of α-crystallin because lenticular proteins are long-lived with negligible turnover and thus subject to various external and internal insults, which can lead

to aggregation of proteins leading to disease. Furthermore, lenticular proteins accumulate several posttranslational modifications (PTMs) such as truncation, deamidation, oxidation, glycation, and racemization during aging and other cataractogenic conditions. Fatefully, α-crystallin has not been immune to these modifications as most of these modifications are shown to alter its chaperone-like activity (Table 1). For instance, truncated α-crystallin isolated from lenses of ICR/f rats, a strain with hereditary cataracts, showed reduced chaperone activity (*64*). Also, deaminated mutants (N146D and N78D/N146D) of αB-crystallin, where asparagines are converted to aspartate, exhibited decreased chaperone activity when compared with wild-type protein (*53*). Similarly, mutations N101D, N123D, and N101D/N123D of human αA-crystallin resulted in attenuation of chaperone activity (*54*). Biophysical and biochemical studies on these deaminated mutants showed that they have alterations in secondary and tertiary structures and formed larger aggregates.

Table 1
Posttranslational modifications and site-directed mutations associated with decreased chaperone activity

Posttranslational modifications	Reference
Deamidation	(*53, 54*)
Glycation	(*55, 56*)
Oxidation	(*57*)
Intradisulfide bonds	(*58*)
Phosphorylation	(*59*)
Racemization	(*60*)
Truncation	(*61*)
Carbamylation	(*62*)
Kynurenination	(*63*)

Formation of intrapolypeptide disulfides during aging contributes to the age-dependent loss in chaperone activity of α-crystallin in human lenses (*65*). Incubation of α-crystallin with oxidized glutathione results in significant loss of its chaperone activity because of the formation of protein–glutathione mixed disulfides (*58*). Recently, we have reported that impairment of chaperone-like activity of α-crystallin by the dementia (ADan) peptides might be the underlying molecular basis for the co-occurrence of cataract with familial Danish dementia (*66*).

The mutation R116C localized to the αA-crystallin gene is responsible for autosomal dominant congenital cataract, which is a common cause of infant blindness (*67*). Correspondingly, the R120G mutation in the αB-crystallin gene is also associated with cataract (*68*). Decreased chaperone activity of recombinant mutants (αA R116C and αB R120G) established the association of α-crystallin chaperone function with cataract (*69, 70*). A missense mutation in αB-crystallin that introduces proline residue at codon 20 to a serine residue (P20S) is associated with autosomal dominant posterior congenital polar cataract (*71*). Subsequently, it was shown that, compared with heteroaggregates of wild-type-αA and -αB, heteroaggregates containing wild-type-αA and P20S αB showed marked decrease in subunit-exchange rate and chaperone activity (*72*). Mutations in αA-and αB-crystallins that associated with hereditary cataract are summarized in Table 2. Knockout studies contributed more evidence to the concept of association of α-crystallin chaperone-like activity with eye lens transparency, as αA-knockout mice developed cataract that started in the nucleus and progressed with age to encompass the whole lens (*81, 83*). An additional interesting finding in these animals was the presence of dense inclusion bodies composed of αB-crystallin (*81*) and γ-crystallin (*84*). From these observations, it becomes evident that αA-crystallin is needed for maintaining the solubility of other crystallins in the lens, including its counterpart αB-crystallin. Moreover,

Table 2
Mutations in αA- or αB-crystallin genes associated with cataract

Protein	Mutation	Remark	Reference
Human αA	R54C	Congenital total white cataract	(*73*)
Human αA	G98R	Peripheral ring-like opacity	(*74*)
Human αA	R21L	Congenital cataract	(*75*)
Human αA	R116C	Autosomal dominant cataract	(*67*)
Human αA	W9X	Autosomal recessive cataract	(*76*)
Human αB	150delA (αB184)	Posterior polar cataract	(*77*)
Human αB	D140N	Autosomal dominant lamellar cataract	(*78*)
Human αB	P20S	Posterior polar cataract	(*71*)
Mouse αA	R54C	Recessive whole cataract	(*79*)
Mouse αA	Y118D	Dominant nuclear cataract	(*79*)
Mouse αA	V124E	Nuclear cataract	(*80*)
Mouse αA	Null mutation	Cataract, micropthalmia	(*81*)
Mouse αA/αB	Double knockout	Whole lens cataract	(*82*)

targeted disruption of both αA and αB-crystallins in mice resulted in development of cataract (*82*). Cataract is considered as a multifactorial disease while aging and diabetes are predominant risk factors (*85*). α-Crystallin chaperone activity was found to be decreased in aged individuals compared with younger subjects and also in aged rats (*34, 44, 45*). It was also noted that chaperone function of α-crystallin has shown to be compromised in diabetic rats and humans (*86–88*). These observations suggest association between decreased chaperone activity and incidence of cataract in diabetic and aged individuals. The major molecular events that are responsible for the development of age-related senile cataract and diabetic cataract are nonenzymatic glycation, activation of polyol pathway, and increased oxidative stress. Elevated activity of aldose reductase and increased production of sorbitol have been implicated for cataractogenesis in diabetic humans and experimental diabetic animals (*89*). Studies from our laboratory and by others have reported that *in vitro* nonenzymatic glycation of α-crystallin resulted in the decreased chaperone activity, which is associated with crosslinking and formation of HMM aggregates (*55, 56, 90, 91*). Moreover, glycation-induced structural changes in α-crystallin are comparable with changes manifested in α-crystallin during diabetic cataract (*87*). Apart from nonenzymatic glycation, several other PTM also affect the chaperone function and are associated with cataractogenesis, such as truncation and oxidation. C-terminal truncation of α-crystallin was observed in diabetic human and rat lenses (*92*) and was associated with decreased chaperone activity. Oxidative stress is considered as an initiating factor for the development of maturity-onset cataract and H_2O_2 is the major oxidant contributing to cataract formation (*93*). α-Crystallin isolated from H_2O_2-treated cultured lenses (*94*) and *in vitro* oxidation of α-crystallin by H_2O_2 and $FeCl_3$ (*90, 95*) resulted in decreased chaperone activity. High Ca^{2+} has been associated with cataract formation by altering the structural stability of α-crystallin and thereby decreasing its chaperone activity (*96*), suggesting a role for this cation in the pathological process. Exposure to UV-radiation is also known as a risk factor for cataract formation, and it should be highlighted that α-crystallin compromise in its chaperone activity on UV-irradiation (*97*).

Diminished chaperone activity of α-crystallin because of mutations, PTM, and its association with development of cataract argue for the importance of α-crystallin chaperone function in eye lens transparency, and draw the attention that attenuation of altered chaperone-like activity of α-crystallin could delay or prevent the cataract formation.

MODULATION OF α-CRYSTALLIN CHAPERONE ACTIVITY

Modulation of α-Crystallin Chaperone Activity by Dietary Agents

Because it is clearly established that α-crystallin chaperone activity is critical for lens transparency, it is surmised that maintaining optimal or higher (increased) chaperone activity might aid in the prevention of cataract. Hence, attempts have been made to modulate or increase the α-crystallin chaperone activity. Studies from our laboratory and elsewhere reported that streptozotocin (STZ) induction has resulted in cataract, and α-crystallin isolated from these cataractous lenses displayed decreased chaperone activity when compared with nondiabetic lenses (*86, 88, 98*). Interestingly, feeding these diabetic rats with a dietary antioxidant, curcumin, prevented the loss of α-crystallin chaperone activity, which is associated with a delay of progression and maturation of cataract (*88, 99*) (Fig. 1). It was shown that oxidative stress along with osmotic stress may be a predominant mechanism in STZ-induced hyperglycemia in rats, and curcumin decreased aldose reductase activity and oxidative stress in diabetic rat lens (*99*). As mentioned earlier, oxidation is known to alter secondary and tertiary structure of α-crystallin as well as oligomeric structure of αA- and αB-crystallins, eventually declining the chaperone activity (*95*). α-Crystallin from diabetic rats showed altered secondary and tertiary structure (*88*). Curcumin feeding to diabetic rats not only attenuated oxidative stress and delayed maturation of cataract (*99*) but also prevented altered tertiary structure and redistribution of α-crystallin in diabetic rat lens (*88*). Thus, it is possible that reduction of oxidative stress as well as inhibition of polyol pathway by curcumin may modulate structural alterations to α-crystallin and prevent the loss of chaperone activity, thereby delay maturation of cataract (*88, 99*).

In contrast to loss of chaperone-like activity in diabetic lens, expression of both αA- and αB-crystallins was elevated in

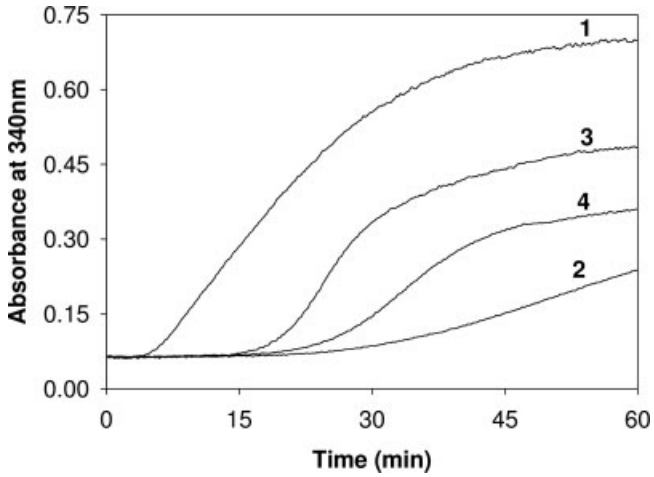

Figure 1. α-Crystallin from curcumin fed diabetic rat showed improved protection than α-crystallin from diabetic rat (*98*). Chaperone activity of α-crystallin was assessed by the suppression of heat-induced aggregation of βL-crystallin. βL-Crystallin was incubated at 65°C in the absence (trace 1) or in the presence of αL-crystallin from control rats (trace 2), diabetic rats (trace 3), and diabetic rat fed with 0.01% curcumin (trace 4).

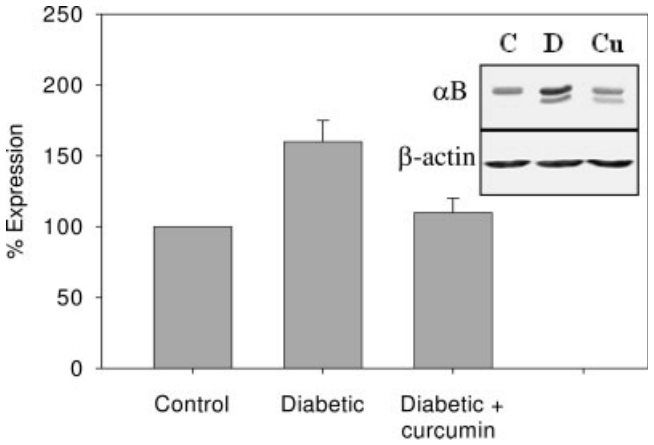

Figure 2. Modulation of αB-crystallin protein in lens of control, diabetic, and diabetic rats fed with 0.01% curcumin. Quantification of αB expression control rat is considered as 100%. Inset-Immunoblot: C, control; D, diabetic; Cu, curcumin treated.

various tissues including the lens of diabetic rats and associated with enhanced degradation or truncation (*14*). Increased oxidative stress, directly or indirectly mediated through enhanced polyol pathway, appears to be a major stimulus for the enhanced expression of αA and αB-crystallins in the tissues of diabetic rats. Interestingly, feeding of curcumin to diabetic rats attenuate the enhanced expression and degradation of α-crystallin subunits (Fig. 2) probably through its antioxidant and aldose reductase inhibitory potential (*14*). Although these studies are preliminary in nature, they provide the scope for future studies to explore the potential of antioxidants and aldose reductase inhibitors for their modulatory role on α-crystallin expression, structure, and chaperone function (*100*). Likewise, prevention of the formation of aggregates induced by αB-crystallin myopathy- and cataract-associated mutants by appropriate means may be an efficient strategy to inhibit the development of the disease. For example, it is well known that the cessation of the expression of αB-crystallin R120G mutant in symptomatic mice improved cardiac function and rescued these animals from premature death (*32*). For example, as reviewed in (*32*), specific peptide/RNA aptamers or chemical chaperones that interfere with the mechanism leading to the aggregation of the mutated αB-crystallin, but not with the wild-type protein functionality, could be a worthwhile strategy and needs further investigations. Importance of compounds that have the potential to modulate specific activities such antioxidant, antiaggregatory, and anti-/proapoptotic function of other sHSp including αA- and αB-crystallin with regard to their involvement in many other diseases is discussed in a recent review (*32*). It would be promising approach to explore the therapeutic ability of these antioxidants of dietary origin against not only cataract but also several other diseases where impaired chaperoning function has been implicated.

Modulation of α-Crystallin Chaperone Activity by Antiglycating Agents In Vitro

Because α-crystallin chaperone activity is shown to be impaired under *in vitro* glycation conditions (*55, 56, 90*), we have also studied the effect of antiglycating compounds, if any, on the α-crystallin chaperone activity under *in vitro* conditions. Interestingly, loss of chaperone activity of α-crystallin due to *in vitro* glycation is prevented because of the presence of aqueous extract of a dietary condiment, cumin, in the reaction mixture because of its antiglycating potential (*98*). Furthermore, we tested the efficacy of antiglycating compound on the modulation of α-crystallin chaperone activity by feeding it to experimentally induced diabetic rats. Feeding cumin to diabetic rats prevented the loss of α-crystallin chaperone activity and also delayed diabetic cataract, and thus substantiated that antiglycating agents may delay or prevent diabetic cataract through minimizing the loss of chaperone activity (*98*).

PTM of bovine α-crystallin by D-erythrose-4-phosphate, fructose-6-phosphate, D-ribose-5-phosphate, and carbamylation resulted in protein crosslinking and HMM aggregates and eventually loss of chaperone-like activity as assessed by γ-crystallin aggregation (*91*). Although ibuprofen prevents crosslinking induced by these modifying agents and prevents loss of α-crystallin chaperone-like activity (*91*), this study suggests that the protective effect of ibuprofen may be exerted by the binding of ibuprofen breakdown products to α-crystallin lysine groups, preventing PTM responsible for the loss of chaperone-like activity. It has been reported that treatment of glucose-6-phosphate glycated αA-crystallin with AGE crosslink breaker, phenacyl-4, 5-dimethylthiazolium bromide resulted in the reversal of loss of chaperone activity due to glycation (*101*). Treatment of H_2O_2-incubated lens with L-carnitine decreased PTMs induced by oxidative stress and safeguarded α-crystallin chaperone activity (*94*). Carnosine, a naturally occurring dipeptide (β-alanyl-L-histidine), was shown to induce the disaggregation of glycated α-crystallin and contribute to the controlled unfolding of glycated protein (*102*). In another study, it was shown that carnosine prevented the fructose-induced crosslinking of α-crystallin in dose- and time-dependent manner, there by prevented the loss of α-crystallin chaperone activity (*103*). Recently, it has been shown that gold nanoparticles prevent the glycation of α-crystallin and formation of AGE by glycating agent fructose (*104*). Therefore, it is reasonable to speculate that if we can prevent the loss of α-crystallin chaperone activity due to glycation that in turn may protect the lens from other insults including glycation.

Modulation of α-Crystallin Chaperone Activity by Small Molecules of Metabolic Interest

Many biological molecules of common metabolic pathways such as pantethine and glutathione have been shown to modulate

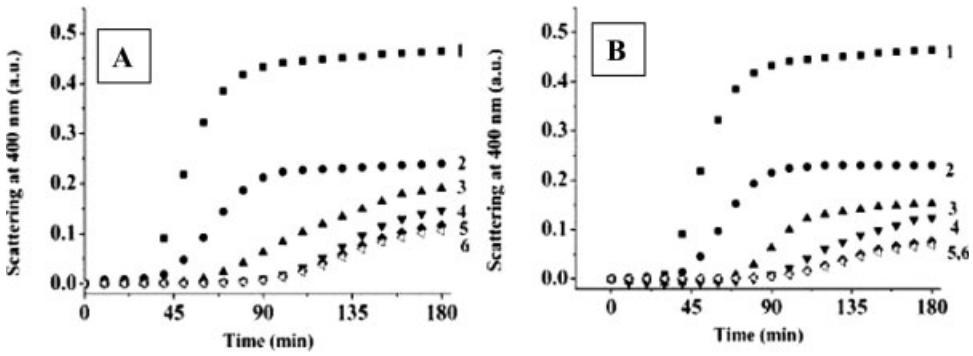

Figure 3. Effect of Zn^{2+} on chaperone activity of αA [A] and αB-crystallin [B] on aggregation of insulin induced by β-mercaptoethanol: (1) insulin, (2) insulin and α-crystallin, (3) insulin, αB-crystallin, and 0.1 mM Zn, (4) insulin, αB-crystallin, and 0.25 mM Zn, (5) insulin, αB-crystallin, and 0.5 mM Zn, (6) insulin, αB-crystallin, and 1 mM Zn. (Adapted from (*109*)).

bovine α-crystallin chaperone activity (*105*). Interesting observation from these studies is that pantethine interacts with α-crystallin and improve its antiaggregation activity in βL-aggregation assay. In contrast, glutathione interacts with alcohol dehydrogenase, which is a substrate in the aggregation assay and resulted in increased chaperone activity of α-crystallin. In another study (*106*), a biologically compatible molecule arginine hydrochloride has been shown to improve chaperone activity of calf α-crystallin and recombinant human αA- and αB-crystallins. This study reports that arginine hydrochloride induces significant changes in quaternary structure and slight changes in the tertiary structure leading to enhanced α-crystallin chaperone activity. Recently, it was shown that chaperone function of αB-crystallin against aggregation of amyloid fibril-forming protein α-synucleinA53T is enhanced in the presence of positively charged small molecules such as arginine, lysine, and guanidine (*107*). It is interesting to note that divalent metal ions have different effect on α-crystallin chaperone activity. Copper and zinc, at concentrations of 0.1 and 1 mM, significantly increase the chaperone-like activity of α-crystallin (*108*). The surface hydrophobicity of α-crystallin was increased by 50% because of the binding of Zn^{2+}, and the stability of α-crystallin was enhanced by 36 kJ/mol, and it became more resistant to tryptic cleavage (*109*). Increased stability and hydrophibicity of α-crystallin could explain the rationale for increased chaperone activity on zinc binding (Fig. 3) (*109*).

Classical chaperones of Hsp family display ATPase activity, and ATP weakens the interaction between chaperone and client protein and thus helps in refolding of client protein. It is fairly believed that sHsp including α-crystallin have no dependence on ATP for their function. Although a study revealed that αA- and αB-crystallins have no ATPase activity (*36*), a weak autophosphorylation activity for α-crystallin has been reported (*110*). It is noteworthy that ATP hydrolysis is not a prerequisite for the refolding of xylose reductase by α-crystallin, presence of ATP enhanced the refolding yield (*111*). In another study, it was reported that ATP enhanced the αB-crystallin ability to reactivate and refolding of citrate synthase (*112*). Furthermore, it has been reported that in the presence of ATP, α-crystallin subunits undergo conformational transition associated with exposure of additional hydrophobic sites and enhanced chaperone activity of α-crystallin (*113*). This study also reveals that ATP induces an enhanced stability of 4.5 KJ/mol of αA-crystallin. Furthermore, binding of ATP to α-crystallin makes more resistant to tryptic cleavage (*113, 114*). These observations suggest that presence of ATP could play a decisive role under conditions which α-crystallin compromise in chaperone function. Incidentally, the concentration of ATP (6 mM) in the lens is one of the highest found among various tissues.

Modification of α-crystallin with metabolic α-dicarbonyl compound, methylglyoxal (MGO), resulted in increased hydrophobicity and enhanced chaperone activity (*115*). It was shown that MGO modification of selective arginine residues (R21, 49, 103) to argpyrimidine makes human αA a better chaperone (*116*). However, the role of MGO in modulating chaperone function of α-crystallin is a subject of debate. Nonenzymatic glycation of α-crystallin with MGO induces crosslinking, aggregation, and increase in molecular size there by reduces its chaperoning ability (*55*). In support to this study, a concentration- and time-dependent decrease in chaperone function of MGO-modified α-crystallin was observed (*117*). In general, chaperone activity of α-crystallin is assessed by *in vitro* aggregation assays and nonaggregation (enzyme inactivation) assays. Although it was observed that MGO-modified α-crystallin display increased chaperone activity in aggregation assays, experimental evidence suggests that it could be due to the caveats of *in vitro* aggregation assays (*55*). However, it should be noted that glycation or AGE formation (including MGO modification) has unequivocally been implicated in the pathogenesis of various pathological complications of diabetes including cataract. Thus, even if the enhanced chaperone-like activity of α-crystallin due to MGO modification is considered to be a true physiological event, the overall effect of MGO-induced crosslinking of lens structural proteins may overwhelm the ability of α-crystallin to suppress aggregation and light scattering.

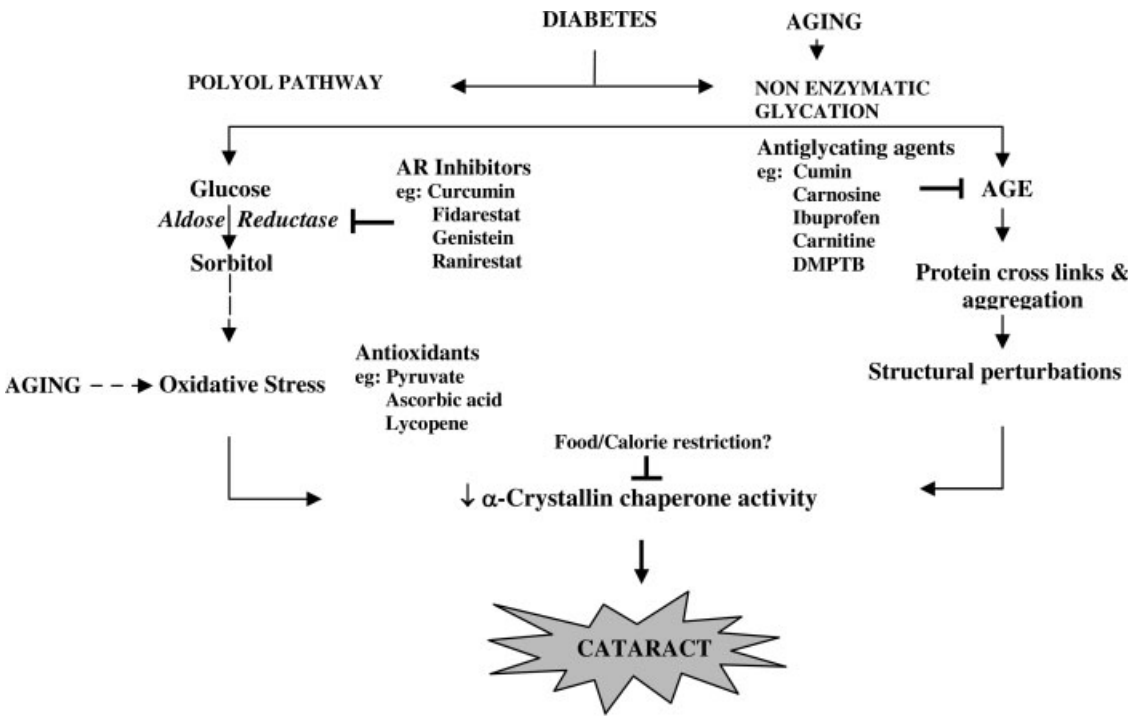

Figure 4. Flow chart describing the possible molecular events that could affect chaperone function of α-crystallin during aging and diabetes and potential target points to modulate α-crystallin chaperone function.

CONCLUSIONS

Several reports describe the importance of α-crystallin chaperone activity in maintaining lens transparency and prevention of cataract. Development of cataract in experimental animals is commonly manifested in the loss of α-crystallin chaperone activity (*88, 98*). Thus, agents that modulate the chaperone activity of α-crystallin subunits merit attention. However, identification of molecules based on the structure of α-crystallin might not be straight forward approach because the three-dimensional structure of α-crystallin is not known. Therefore, alternative approach is to identify the factors or molecular events that affect the chaperone activity of α-crystallin and explore the compounds that modulate those events. Several etiological factors and molecular events are responsible for cataract formation, such as nonenzymatic glycation, increased sorbitol production via aldose reductase pathway, and oxidative stress. It appears that these events both individually or cumulatively could affect chaperone function of α-crystallin and end up in the development of cataract, and thus these pathways become obvious targets of modulation α-crystallin chaperone function (Fig. 4). Studies revealed that the inhibitors of aldose reductase and nonenzymatic glycation delayed cataract progression/maturation under diabetic condition (*98, 99*). Incidentally, delay in diabetic cataract in the rats fed with aldose reductase inhibitor or antiglycating agent coincided with positive modulation of α-crystallin chaperone activity (*88, 98*). Therefore, it appears that modulation of α-crystallin chaperone activity could evade the cataract progression. It has been estimated that at least a 10-year delay in cataract incidence will decrease the number of cataract surgeries by 45%. It should be noted that besides antiglycating agents, antioxidants and aldose reductase inhibitors, a variety of agents such as divalent metal ions, arginine hydrochloride, glutathione, pantethine, and carnosine have been associated with modulation of α-crystallin chaperone activity (Table 3). This argues for a combination of major anticataractous agents, as a

Table 3
Agents reported to modulate chaperone-like activity of α-crystallin

Agent	Reference
Curcumin—antioxidant/aldose reductase inhibitor	(*88*)
Cumin—antiglycating agent	(*98*)
Glutamine, pantethine—metabolites	(*105*)
Carnitine—endogenous derivative of amino acids	(*94*)
Carnosine—dipeptide	(*102*)
Zinc—increase the stability and hydrophobicity	(*109*)
Ibuprofen-NSAID	(*91*)
Arginine-HCl	(*106*)
Lysine and arginine	(*107*)
Phenacyl-4,5-dimethylthiazolium bromide (DMPTB)	(*101*)

dietary supplementation could be a promising approach. The ability of small molecules such as glutathione, lysine, or arginine to modulate the chaperone activity of α-crystallin seeds a concept of using these molecules as minichaperons to facilitate efficient protein folding.

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