Alpha-crystallin–derived peptides as therapeutic chaperones

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Abstract

Background—The demonstration of chaperone-like activity in peptides (mini-chaperones) derived from α-crystallin's chaperone region has generated significant interest in exploring the therapeutic potential of peptide chaperones in diseases of protein aggregation. Recent studies in experimental animals show that mini-chaperones could reach intended targets and alter the disease phenotype. Although mini-chaperones show potential benefits against protein aggregation diseases, they do tend to form aggregates on storage. There is thus a need to fine-tune peptide chaperones to increase their solubility, pharmacokinetics and biological efficacy.

Scope of Review—This review summarizes the properties and the potential therapeutic roles of mini-chaperones in protein aggregation diseases and highlights some of the refinements needed to increase the stability and biological efficacy of mini-chaperones while maintaining or enhancing their chaperone-like activity against precipitation of unfolding proteins.

Major conclusions—Mini-chaperones suppress the aggregation of proteins, block amyloid fibril formation, stabilize mutant proteins, sequester metal ions and exhibit antiapoptotic properties. Much work must be done to fine-tune mini-chaperones and increase their stability and biological efficacy. Peptide chaperones could have a great therapeutic value in diseases associated with protein aggregation and apoptosis.

General significance—Accumulation of misfolded proteins is a primary cause for many age-related diseases, including cataract, macular degeneration and various neurological diseases. Stabilization of native proteins is a logical therapeutic approach for such diseases. Mini-chaperones, with their inherent antiaggregation and antiapoptotic properties, may represent an effective therapeutic molecule to prevent the cascade of protein conformational disorders. Future studies will further uncover the therapeutic potential of mini-chaperones.

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1. Introduction

The primary function of the eye lens is to focus light on the retina. The lens is composed mostly of specialized proteins called crystallins [1]. The three main types of lens crystallins are α-, β- and γ-crystallin, which account for nearly 90 percent of the lens proteins. α-Crystallin is the predominant type [2] and is composed of two types of subunits, A and B, which non-covalently associate to form aggregates with an average molecular mass of 800 kDa. Both αA- and αB-crystallin subunits have chaperone-like activity that helps maintain the lens clarity [3, 4]. The other two crystallins, β- and γ-crystallin, have no chaperone activity and serve as structural proteins. While lens proteins have very little turnover, they do undergo age-related post-translational modifications that lead to aggregation [1, 4]. The age-related changes affect the interactions of α-, β- and γ-crystallin that play a role in the modulation of lens clarity. The αA-crystallin subunit is more prevalent than the αB subunit, in a ratio of 3:1 to 2:1 [4, 5]. The primary structures of αA- and αB-crystallin subunits exhibit a high degree of sequence similarity between them and to small heat shock proteins (sHSPs) because of the conserved α-crystallin domain in these proteins [6]. The secondary structure of α-crystallin is primarily in the form of β sheets. The tertiary and quaternary structure of α-crystallin is becoming clearer with the application of modern techniques to study the oligomeric structure of the protein [7–9]. Nuclear magnetic resonance studies show that the C-terminal regions of αA- and αB-crystallins are more flexible than the N-terminal regions [10]. The α-crystallin molecule is a dynamic oligomer, with the subunits dissociating and reassociating constantly. Under in vitro conditions, the subunits of the α-crystallin molecule exchange with a new set of subunits in about 4 hours [11]. The binding of partially unfolded β- or γ-crystallins to either of the α-crystallin subunits affects the subunit exchange [12–14]. Proper subunit interaction between αA- and αB-crystallin is clearly necessary to prevent the formation of light scattering aggregates of crystallins, an initial age-related change in the lens that could be the first event in the development of cataracts [13].

2. α-Crystallin chaperone activity and lens transparency

α-Crystallin chaperone activity, first discovered in 1992 by Horwitz [15], is thought to be involved in maintaining lens clarity. α-Crystallin (or its subunits) has been shown to suppress the aggregation of proteins partially unfolded by oxidation, heat and other stressors [16–18]. Cells expressing α-crystallin have increased thermoresistance [19]. α-Crystallin chaperone activity is modulated by low-molecular-weight compounds such as adenosine triphosphate (ATP) and glutathione, the concentrations of which are known to change in the lens with aging [20]. Modification of α-crystallin by glycation, ultraviolet irradiation and deamidation leads to the loss of chaperone activity [12, 21, 22]. The general consensus is that in addition to the structural role of α-crystallin in the lens, α-crystallin also functions as a chaperone in vivo. The factors that suppress α-crystallin chaperone function are thought to accelerate aggregation of other crystallins that are undergoing age-related modifications and losing their native structure. Consistent with this hypothesis, aged human lenses indeed exhibit decreased chaperone activity, increased crystallin aggregation, light scattering and loss of lens transparency [22–24]. Studies of total lens homogenates and whole lenses, as

well as αA knockout studies and congenital cataract studies, also argue in favor of the importance of α-crystallin chaperone function in the lens [25–29].

Multiple regions in α-crystallin subunits may be involved in chaperone activity [30–33]. Studies of congenital cataracts suggest that the structural changes in the mutant proteins expressed in congenital cataracts are likely to 1) mask the chaperone site and prevent chaperone action, 2) interfere with αA- and αB-crystallin subunit interactions, or 3) make the chaperone protein "hyperactive" and unstable, resulting in a chaperone–substrate complex that aggregates [18, 28, 34–36]. A number of studies have shown that the structure of α-crystallin must remain dynamic, with subunits constantly dissociating and reassociating, in order for chaperone activity to be maintained [13, 14, 37]. In support of this is an earlier study that showed recovery of chaperone activity in crystallins after cleavage of chemically cross-linked protein [38]. However, Augusteyn reported that glutaraldehyde cross-linked crystallin shows marginally higher chaperone activity than the native protein and attributed the maintenance of surface interaction between crystallin and client protein to preservation of chaperone activity [39].

3. Identification of chaperone sites in α-crystallin

Soon after chaperone activity of α-crystallin was reported in 1992, several investigators initiated studies to uncover the sequences/regions in the protein responsible for the activity. The C-terminal region [40], αA69D [41], the phenylalanine-rich N terminal region [42] and αB-R120 [43] were all shown to be important for the chaperone-like activity in α-crystallin subunits. Similar to the activity of the GroEL system [44], a strong correlation was found to exist between the exposed hydrophobic surface of the protein and chaperone activity of α-crystallin subunits [44–46]. Early studies demonstrated that heat treatment of α-crystallin subunits leads to increased exposure of hydrophobic sites and that the heat-treated crystallin exhibits increased chaperone activity. Other methods of perturbation of the α-crystallin oligomer structure and increased exposure of hydrophobic patches also result in enhanced chaperone activity [47, 48].

Nearly 20 years ago, we set out to begin mapping the hydrophobic sites responsible for chaperone activity. We used the hydrophobic probe bis-ANS, photo-crosslinking and amino acid sequencing methods to identify the residues located at the chaperone site [30, 49]. We found that bis-ANS interacts with residues 50–54 and 79–99 in αA-crystallin and with residues 75–103 in αB-crystallin [30, 50]. In related studies we showed that melittin peptide interacts with α-crystallin [31] and that this interaction interferes with the chaperone function of crystallin [31, 51]. Therefore, we used novel cross linkers to cross-link melittin to αA-crystallin and to determine the melittin interaction sites on crystallin. Our approach led to the identification of $_{12}$RTLGPFYPSR$_{21}$ and $_{70}$KFVIFLDVKHFSPEDLTVK$_{88}$ sequences in αA-crystallin as mellitin-binding sites. Reasoning that the hydrophobic site and the melittin interaction site on αA-crystallin were one and the same, we synthesized a 19 amino acid peptide that corresponds to the 70–88 sequence (KFVIFLDVKHFSPEDLTVK) in αA-crystallin and demonstrated that this peptide encapsulates the chaperone activity of αA-crystallin [31]. Based on our hydrophobic site studies, on the client protein alcohol dehydrogenase (ADH) binding studies [52, 53] and on the sequence similarity between αA-

and αB-crystallin, we synthesized a 20 amino acid peptide (DRFSVNLDVKHFSPEELKVK), corresponding to the 73–92 region in αB-crystallin, and with this peptide showed that the 73–92 region is the chaperone site in αB-crystallin [51]. We named the synthetic peptides exhibiting chaperone activity "mini-chaperones." In various publications they have been also called "αA-mini-chaperone," "mini-αA peptide" and "mini-αB peptide," etc. Using pin-array studies, Ghosh and Clark identified additional chaperone peptides representing specific regions of αB-crystallin: $_{73}$DRFSVNLDVKHFS$_{85}$, $_{113}$FISREFHR$_{120}$, $_{131}$LTITSSLSDGV$_{142}$ and $_{156}$ERTIPITRE$_{164}$ [54, 55]. Of the chaperone peptides identified by pin-array studies, one peptide, $_{73}$DRFSVNLDVKHFS$_{85}$, was found to overlap with the mini-chaperone sequence identified earlier [51].

4. Synthetic chaperone peptides derived from mini-αA- and mini-αB-crystallins

Our studies established that both mini-αA70-88 (KFVIFLDVKHFSPEDLTVK) and mini-αB73-92 (DRFSVNLDVKHFSPEELKVK) peptide chaperones are effective in preventing aggregation and precipitation of unfolding proteins, similar to the full-length native α-crystallin subunits [31, 51]. The mini-chaperone sequence is highly conserved across many small heat shock proteins (Table 1) and aligns with the β3 and β4 region in a three-dimensional (3D) crystal structure of truncated α-crystallin [56, 57]. Substitution of Lys with Asp in the mini-αA-peptide increases the peptide's solubility and chaperone-like activity. We have investigated the minimum sequence of the peptide essential for chaperone activity, with the intent of developing efficient and powerful chaperone(s) that can be used widely for therapeutic purposes, including for cataract, neurodegenerative diseases and other protein aggregation disorders. Table 2 lists several αA-crystallin–derived peptides with varying degrees of chaperone activity. We found that truncation of the KFVIF sequence at the N-terminal region of mini-αA70-88 (mini-αA75-88) completely abolished the chaperone activity. The removal of VK from the C-terminus of mini-αA (mini-αA 87-88) does not affect chaperone function. However, removal of DLTVK from the C-terminal end of the peptide (mini-αA70-83) completely impairs the chaperone function [31]. Further, we found that substitution of F71 with Arg (mini-αA-F71R) results in complete loss of its function, indicating the importance of aromatic residue Phe at the 71st position in αA-crystallin. Phe71 is a highly conserved residue in sHSPs, across many species (Table 1). It is well documented that the addition of charged amino acids to the peptides can produce dramatically different levels of activity [58]. We also attempted to add additional residues at either the N- or C-terminal, or both termini, to create chaperone peptides with increased activity. The charged amino acids, either DD or RR, were fused at the C-terminal end, and the new peptides with additional charged residues DFVIFLDVKHFSPEDLTVKDD and DFVIFLDVKHFSPEDLTVKRR showed total impairment of chaperone function. The addition of RG residues at the N-terminal and GR residues at the C-terminal end (RG-mini-αA-GR) resulted in a 50% loss in chaperone activity, whereas addition of VQED residues at the C-terminal (mini-αA-VQED) retained the chaperone activity of the original mini-αA peptide. We also added a mixture of charged residues (neutral, positively and negatively charged residue GRD) at the C-terminal end of mini-αA. To our surprise, the resulting

peptide, DFVIFLDVKHFSPEDLTVKGRD (mini-αA-GRD), showed significantly higher (25%) chaperone activity than the original mini-αA peptide (Table 2).

In 2008 a correlation was first reported between chaperone-like activity and fibril formation of αA-crystallin–derived peptides [59]. In our investigations of fibril formation, we found that shaking the mini-αA-chaperone at 900 rpm for several hours leads to amyloid fibril-like aggregates. We also found that αA-crystallin–derived peptides αA66-80 and αA67-75 readily form fibrils at physiological conditions, leading to our suggestion that the αA67-75 region is the driving force in amyloid-like aggregate formation by αA-crystallin. The αA66-80 and αA67-75 peptides contain the core sequence FVIFLD, which has sequence similarity to the β-amyloid region involved in fibril formation [60]. Studies have shown that lengthening the fibril-forming peptides disrupts the propensity to form fibrils [61, 62]. A high degree of flexibility (and lack of structure) of the C-terminal extension in αA-crystallin (EEKPTSAPSS) was identified by nuclear magnetic resonance (NMR) [10]. When this region is appended to the C-terminal end of the mini-chaperone region, it would therefore be expected to have a significant effect in preventing association to form fibrils. To overcome the fibril-forming property of mini-αA, we added the hydrophilic water-soluble C-terminal extension "EEKPTSAPSS" of αA-crystallin to the mini-αA peptide. Our studies revealed that the chimeric peptide (DFVIFLDVKHFSPEDLTVKEEKPTSAPSS, CP1) retains chaperone-like activity and displays a strong resistance to fibril formation [61]. However, there was no gain of chaperone activity as compared to the original mini-αA peptide. Table 3 lists the peptides we tested for chaperone-like properties in mini-αB chaperone. None of the modified peptides showed better chaperone function than the original mini-αB sequence against partially unfolded ADH. We found that deletion of DRFS residues from the N-terminal side (mini-αB77-92) or deletion of LKVK from the C-terminal side (mini-αB73-88) of mini-αB abolishes the chaperone function against partially unfolded ADH.

5. Mini-αA-crystallin prevents proteins aggregation and fibril formation and rescues mutant crystallin

In the last 15 years, we have tested the mini-chaperone in different ways to compare its activity with that of native protein. These experiments investigated the mini-chaperone's ability to prevent aggregation of ADH and citrate synthase (CS) partially unfolded by heat, to prevent ultraviolet light–induced γ-crystallin aggregation, and to prevent dithiothreitol (DTT)–induced insulin and α-lactalbumin aggregation [31, 63, 64]. Our studies revealed that mini-αA-crystallin binds to reduced α-lactalbumin at 2:1 ratios (2 parts mini-αA and 1 part α-lactalbumin) [64]. The circular dichroism (CD) spectra of the mini-αA–α-lactalbumin complex shows loss of α-helix but preserves the β-sheet content, suggesting that mini-αA not only binds to the reduced α-lactalbumin but also maintains the molten-globule state of the reduced lactalbumin in the complex [64]. We also found that the mini-αA peptide prevents fibril formation and toxicity of Aβ-amyloid peptide [65]. We have also identified a peptide (αA66-80) derived from αA-crystallin that promotes protein aggregation and forms fibril-like structures similar to Aβ-amyloid. In our ADH aggregation assays to determine whether the mini-αA suppresses the protein aggregation properties of αA66-80 peptide, we found that the rapid ADH aggregation in the presence of αA66-80 peptide is significantly

reduced in the presence of mini-αA, suggesting that mini-αA peptide can counteract the effect of αA66-80 peptide (Figure 1). Similar to mini-αA peptide, mini-αB peptide was found to prevent DTT-induced insulin aggregation [51]. Further, other investigators have found that mini-αB73-92 forms fibrils and is an effective therapeutic molecule for experimental autoimmune encephalomyelitis [66]. K90-V100 in αB-crystallin has been shown to be highly amyloidogenic and cytotoxic and its crystal structure has been determined [67].

The cataract-causing αA-crystallin mutant protein (i.e., αA-G98R) is prone to aggregation at physiological conditions and fails to chaperone the precipitation of unfolding ADH and insulin. We tested the effect of mini-αA on the aggregation of αA-G98R-crystallin and found that mini-αA stabilizes the mutant protein from aggregation and rescues the lost chaperone function [68]. In other studies, both mini-αA and mini-αB have been shown in mice to inhibit selenite-induced cataract [69]. The mechanism by which αA- and αB-crystallin interact with their client proteins is not yet elucidated because of the difficulty in delineating the structure of a large protein complex involving crystallin oligomer and client protein, but the availability of mini-chaperone allows investigation of the mechanism of αA-crystallin chaperone function. In a recent study of the molecular mechanisms of mini-αA chaperone function, using the NMR method and γD-crystallin as the client protein, mini-αA was found to bind with Phe56, Val132, residues in the Val164 to Leu167 region and along the domain interface of γD-crystallin, expanding our understanding of the molecular mechanisms of mini-αA chaperone function and its potential as a therapeutic molecule [70]. Understanding the critical residues involved in chaperone function in mini-chaperone will allow one to design synthetic mimics of mini-chaperones with greater stability.

6. Antioxidant action of mini-αA-chaperone

Antioxidant molecules play an important role in cell biology and human health, preventing the accumulation of free radicals and stopping cell damage and cell death mediated by free radicals. Previous studies have shown that lens α-crystallins bind Cu(II) and prevent the formation of Cu(II)-mediated reactive oxygen species [71–73]. Mini-αA chaperone indeed functions like an antioxidant by preventing Cu(II)-mediated oxidation of ascorbic acid [74]. We have shown that each molecule of mini-αA binds one Cu(II) molecule, based on the isothermal titration calorimetry (Kd 10.72 μM) and nanospray mass spectrometry (Kd 9.9 μM). Histidine was found to be a critical residue of mini-αA-crystallin involved in Cu(II) binding. Substitution of His with Ala in the peptide (corresponding to residue 79 in native protein) eliminates the redox-suppression activity. Interestingly, mini-αA mimics the redox-suppression activity of native α-crystallin and this property of crystallin is in contrast to the Aβ-amyloid interaction with Cu(II) where H_2O_2 is generated [75].

7. Antiapoptotic property of mini-chaperones

Apoptosis is a cascade of events culminating in programmed cell death. It is well established that α-crystallin functions like an antiapoptotic agent and prevents apoptosis-mediated cell death [28, 76]. Studies of human fetal retinal pigment epithelial cells (hfRPE), challenged with H_2O_2 in the presence and absence of mini-αA- and mini-αB-crystallins, have revealed

that the chaperone peptides function like antiapoptotic agents [77]. Both mini-αA and mini-αB peptides were found to prevent oxidation-induced cell death by inhibiting caspase-3 activation. To investigate whether the antiapoptotic property of mini-chaperone is applicable to other cell lines subjected to oxidative stress, we challenged COS7 and ARPE-19 cells with H_2O_2 in the presence and absence of the mini-αA peptide. We found that the mini-chaperone protects both COS-7 and ARPE-19 cells from H_2O_2-mediated cell death (P<0.01) as compared to COS7 and ARPE-19 cells not treated with mini-αA peptide [61]. Other investigators recently demonstrated in a mouse model of retinal degeneration that mini-αA protects RPE cells from sodium iodate ($NaIO_3$)-induced cell apoptosis and retinal degeneration [78]. Together these results suggest that the natural protective effect of α-crystallin chaperone proteins is encapsulated in the peptide chaperones and may offer an opportunity to use mini-chaperones as pharmacologic agents.

8. Therapeutic value of mini-chaperones

The many studies carried out thus far show that both αA- and αB-mini-chaperones possess the chaperone activity of the parent proteins and are potential candidates for therapeutic applications in a variety of conditions. αA- and αB-mini-chaperones may offer protection to the retina. One study revealed that hfRPE cells uptake the mini-chaperone peptides rapidly via sodium-coupled oligopeptide transporters 1 and 2 and protect the cells from apoptosis induced by oxidative stress [77]. In a related study retinal degeneration caused by $NaIO_3$ was prevented by mini-αA-chaperone [78]. In another study, the peptide chaperone injected intraperitoneally was shown to enter into the blood circulation and cross the blood plasma membrane to protect the lens from selenite-induced cataract [69]. Furthermore, intraperitoneally injected mini-αB-peptide was found to improve clinical symptoms in experimental autoimmune encephalitis [66]. A more detailed account of therapeutic value of peptide chaperone is provided elsewhere in this issue.

9. The challenges facing the development of peptide chaperones and alternatives

While synthetic peptides have been widely proposed for the treatment of different diseases, figuring out a way to deliver the peptides into tissues and circumvent the short half-life of peptides in the bloodstream (due to the presence of exo- and endopeptidases) is quite a challenge to the development of peptide chaperones as effective therapeutic molecules [79, 80]. Harnessing the power of nanoparticles may be an approach. A recent study of a nanoparticle-engineered mini-chaperone peptide, mini-αB73-92, with a high-molecular-weight carrier, showed that the nanoparticle-engineered mini-chaperone provides enhanced protection from oxidative stress and resides for a longer time in retinal cells [81]. Certain modifications of amino acids are known to offer resistance to peptidase action. For example, use of acetylated amino acids is a well-studied modification that provides resistance to peptidase action. Acetylation of mini-chaperone peptide was found to enhance the mini-chaperone's resistance to degradation and improve its efficiency in inhibiting stress-induced apoptosis in human lens epithelial cells and in Chinese hamster ovary cells [82]. In another study of approaches to enhancing the properties of mini-chaperones, substitution of a single Asp isomer (D-Asp) was found to alter significantly the activity of αA70-88 peptide [83].

We synthesized a mini-chaperone peptide with D-amino acids to test whether a peptide resistant to peptidases retains its chaperone activity and found that D-amino acids enhance the chaperone activity of mini-αA by two-fold but not mini-αB (Figure 2). Taken together the data clearly show that mini-chaperones could be made more resistant to in vivo degradation and this approach might provide additional opportunities to test peptide mini-chaperones as disease-modifying agents.

10. Concluding remarks

During the last 15 years, it has been well documented that the mini-chaperone peptide is a potential candidate molecule for therapeutic use in diseases associated with protein aggregation. The concept of mini-chaperones is evolving rapidly, as evidenced by the ever-increasing number of published studies in this field. The retention of biological activity by the mini-chaperones administered intraperitoneally suggests that use of the peptides to treat specific conditions is a viable option [69]. Biotechnology companies have started marketing mini-chaperone peptides for therapeutic studies. Several strategies are under investigation to optimize mini-chaperone peptides, including the addition of non-natural D-amino acid and modification of selective residues. Future studies on the effects of mini-chaperone peptides in various animal models of proteinopathies will enable the development of mini-chaperone peptides as therapeutic agents.

Acknowledgments

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Highlights

Peptide chaperone derived from α-crystallin has therapeutic potential.

Fine-tuning of peptide chaperone is required to increase stability and biological efficacy.

Peptide chaperones could have a great therapeutic value in diseases associated with protein aggregation and apoptosis.

Fig 1.
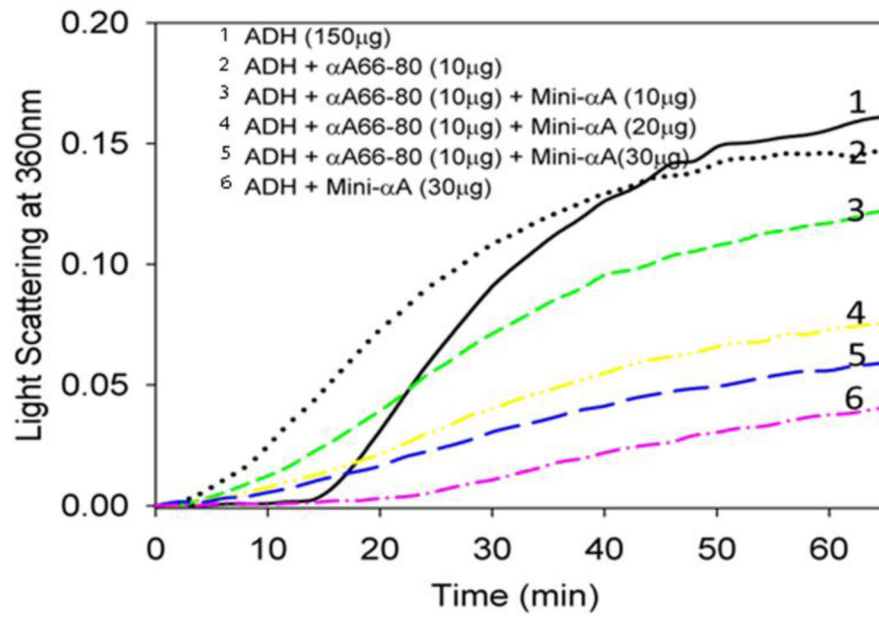The efficacy of mini-αA chaperone on αA66-80 peptide accelerated ADH aggregation. (1) ADH 150 μg. (2) ADH 150 μg and αA66-80 10 μg. (3) ADH 150 μg and αA66-80 10 μg and mini-αA 10 μg. (4) ADH 150 μg and αA66-80 10 μg and mini-αA 20 μg. (5) ADH 150 μg and αA66-80 10 μg and mini-αA 30 μg. (6) ADH 150 μg and mini-αA 10 μg. The chaperone activity was measured using the method described earlier [61]

Fig 2.
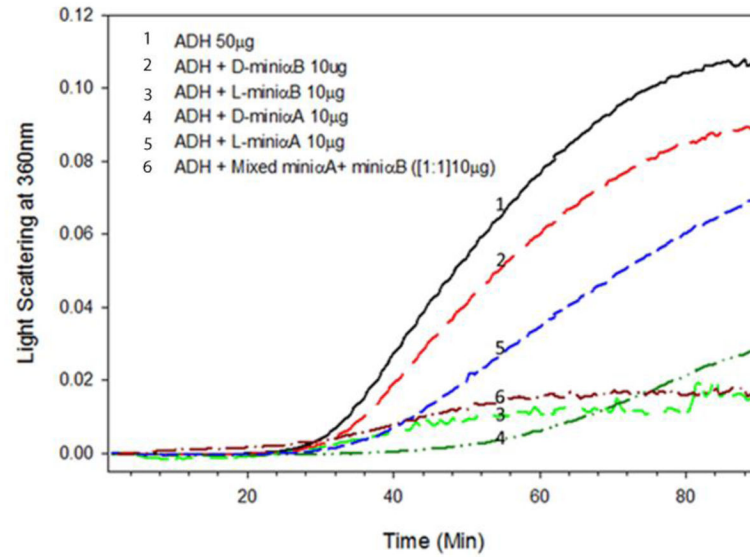Comparison of mini chaperone activities; synthesized with D-amino acid or L-amino acid. ADH aggregation assay was performed to test the efficacy of chaperone like function of mini chaperone's L and D form. (1) ADH 50 μg. (2) ADH 50 μg and D-mini-αB 10 μg. (3) ADH 50 μg, L-mini-αB 10 μg. (4) ADH 50 μg, D-mini-αA 10 μg, (5) ADH 50 μg, L-mini-αA 10 μg. (6) ADH 50 μg, mixed D-mini-αB and D-mini-αA (1:1) 10 μg.

Table 1

Comparison of chaperone region sequences of α-crystallin with various sHsps.

Protein	GenBank	Sequence
Homo sapiens [Human] αA-crystallin	AAC50900	069 DKFVIFLDVKHFSPEDLTVK 088
Homo sapiens [Human] αB-crystallin	NP_001876	073 DRFSVNLDVKHFSPEELKVK 092
Homo sapiens [Human] HspB2	Q16082.2	072 GKFQAFLDVSHFTPDEVTVR 091
Homo sapiens [Human] HspB3	Q12988.2	069 SHFQILLDVVQFLPEDIIIQ 088
Homo sapiens [Human] HspB9	Q9BQS6.1	052 DGFQMKLDAHGFAPEELVVQ 071
Mus musculus [House mouse] αB-crystallin	AAH10768	073 DRFSVNLDVKHFSPEELKVK 092
Rattus norvegicus [Norway rat] αB-crystallin	NP_037067	073 DRFSVNLDVKHFSPEELKVK 092
Bos taurus [Cow] αB-crystallin	AAB95323	073 DRFSVNLDVKHFSPEELKVK 092
Sus scrofa domestica [Domestic pig] αB-crystallin	JC5971	073 DRFSVNLDVKHFSPEELKVK 092
Gallus gallus [Chicken] αB-crystallin	NP_990507	072 DKFSVNLDVKHFSPEELKVK 091
Rana catesbeiana [Bullfrog] αB-crystallin	CAA60594	071 DKFSINLDVKHFSPEELKVK 090
Clarias batrachus [Walking catfish] αB-crystallin	AAO24775	070 DRFTINLDVKHFTPEELGVK 089
Danio rerio [Zebrafish] αB-crystallin	AAD49096	070 DRFVINLDVKHFSPDELTVK 089
Squalus acanthias [Spiny dogfish] αB-crystallin	CYDFAB	075 DKFAIHLDVKHFTPEELRVK 094
Bombyx mori [domestic silkworm] Hsp20.8	AAG30944	073 DKFQVNLDVQHFSPEEISVK 092
Astyanax mexicanus [Blind cavefish] αA-crystallin	CAA72158	070 DKFMVYLDVKHFSPEELNVK 089
Homo sapiens [Human] HspB6	AAH68046	072 GHFSVLLDVKHFSPEEIAVK 091
Columba livia [Domestic pigeon] αA-crystallin	CAA65411	058 EKFTIMLDVKHFSPEDLSVK 077
Lonomia oblique Hsp3	AAV91362	070 DKFQVNLDVQHFAPEEIAVK 089
Bombyx mori [domestic silkworm] Hsp23.7	BAD74198	088 DKFQINLDVQHFSPDEISVK 107
Caenorhabditis elegans Hsp16.41	AAF60616	052 SKFSVQLDVSHFKPENLKIK 071
Danio rerio [Zebrafish] Hsp25	AAV97950	100 DSWKISLDVNHFSPEELNVK 119
Oryzias latipes [Japanese medaka] αA-crystallin	CAA04397	052 DKFTVHWDVKHFSPDELSVK 071
Ostertagia ostertagi Hsp20	CAG25499	062 KKFAVALDVSHFRPEELKVQ 081
Mus musculus [House mouse] Hsp27	AAA18335	096 DRWRVSLDVNHFAPEELTVK 115
		* *** * **** *

The alignment was generated by ClastalW2 multiple sequence alignment using default settings. Residues that are highly conserved across the species are identified by * at the bottom of the Table.

Table 2

Comparison of chaperone activity of mini-αA-crystallin and its variants.

Peptide name	Peptide Sequence	PI	Client Protein	Relative chaperone Activity[1]	Ref
Mini-αA70-88	KFVIFLDVKHFSPEDLTVK	6.7	ADH	95	31
Mini-αA(F71Y)	KYVIFLDVKHFSPEDLTVK	6.7	ADH	26	31
Mini-αA(F71G)	KGVIFLDVKHFSPEDLTVK	6.7	ADH	15	31
Mini-αA(F71R)	KRVIFLDVKHFSPEDLTVK	8.5	ADH	0	31
Mini-αA(F71D)	KDVIFLDVKHFSPEDLTVK	5.4	ADH	25	31
Mini-αA70-83	KFVIFLDVKHFSPE	6.7	ADH	0	31
RD-Mini-αA70-83	RDKFVIFLDVKHFSPE	6.7	ADH	0	31
Mini-αA71-88	FVIFLDVKHFSPEDLTVK	5.3	ADH	75	31
Mini-αA72-88	VIFLDVKHFSPEDLTVK	5.3	ADH	8	31
Mini-αA75-88	LDVKHFSPEDLTVK	5.3	ADH	0	31
Mini-αA	DFVIFLDVKHFSPEDLTVK	4.6	ADH	100	31
Mini-αA 87-88	DFVIFLDVKHFSPEDLT	4.2	ADH	100	PR
Mini-αA 83-88	DFVIFLDVKAFSP	4.2	ADH	0*	PR
Mini-αA(I73P)	DFVPFLDVKHFSPEDLTVK	4.6	ADH	0	PR
Mini-αA extended 70-96	DFVIFLDVKHFSPEDLTVKVQEDFVEI	4.1	ADH	0	61
Chimeric peptide (CP1)	DFVIFLDVKHFSPEDLTEEKPTSAPSS	4.3	CS	85	61
CP1- 172-173	DFVIFLDVKHFSPEDLTEEKPTSAP	4.3	CS	25	61
CP1- 170-173	DFVIFLDVKHFSPEDLTEEKPTS	4.3	CS	10	61
CP1- 168-173	DFVIFLDVKHFSPEDLTEEKP	4.3	CS	15	61
CP1- 166-173	DFVIFLDVKHFSPEDLTEE	4.0	CS	50	61
Mini-αA70-86 GR	DFVIFLDVKHFSPEDLTGR	4.4	ADH	75	PR
Mini-αA GR	DFVIFLDVKHFSPEDLTVKGR	4.6	ADH	80	PR
Mini-αA GRR	DFVIFLDVKHFSPEDLTVKRR	5.4	ADH	0*	PR
Mini-αA GDD	DFVIFLDVKHFSPEDLTVKDD	3.9	ADH	0*	PR
Mini-αA GRD	DFVIFLDVKHFSPEDLTVKGRD	4.3	ADH	125	PR
Mini-αA 87-88 GRR	DFVIFLDVKHFSPEDLTGRR	5.4	ADH	0*	PR
Mini-αA 87-88 GDD	DFVIFLDVKHFSPEDLTGDD	3.9	ADH	50	PR
Mini-αA 87-88 GRD	DFVIFLDVKHFSPEDLTGRD	4.3	ADH	110	PR

Peptide name	Peptide Sequence	PI	Client Protein	Relative chaperone Activity[1]	Ref
RGMini-αAGR	RGDFVIFLDVKHFSPEDLTVKGR	6.7	Insulin	50	PR
Mini-αAVQED	DFVIFLDVKHFSPEDLTVKVQED	4.2	ADH	90	PR
DQ Mini-αA GRD	DQFVIFLDVKHFSPEDLTVKGRD	4.7	ADH	75	PR
Mini-αA Cys	DFVIFLDVKHFSPEDLTVKC	4.6	ADH	100	PR
Cys Mini-αA	CDFVIFLDVKHFSPEDLTVK	4.6	ADH	95	PR
Mini-αA retro	KVTLDEPSFHKVDLFIVFD	4.6	Insulin	0	84
Mini-αA (all D forms)	DFVIFLDVKHFSPEDLTVK	4.6	ADH	130	PR
RG Mini-αA (76 D-Asp) 85,87 GR	RGFVIFL{D-Asp}VKHFSPEDTKGR	8.6	ADH	80	PR
RG Mini-αA (76 D-Asp) 87-88 GR	RGFVIFL{D-Asp}VKHFSPEDLTGR	6.7	ADH	85	PR
Mini-αA (76 D-Asp) 85,87 GR	DFVIFL{D-Asp}VKHFSPEDTK	4.6	ADH	80	83

The residues shown in color are derived from αA-crystallin chaperone region (αA70-88).

*
indicates peptide by itself aggregated. PR–present study

[1]The chaperone activity of peptides was compared against Mini-αA. The chaperone activity of mini-αA was considered 100.

Table 3

Comparison of chaperone activity of mini-αB-crystallin and its variants.

Peptide name	Peptide Sequence	PI	Client Protein	Relative Chaperone activity[1]	Ref
Mini-αB 73-92	DRFSVNLDVKHFSPEELKVK	6.7	ADH	100	51
Mini-αB 73-90	DRFSVNLDVKHFSPEELK	5.4	ADH	64	51
Mini-αB 73-88	DRFSVNLDVKHFSPEE	4.7	ADH	0	51
Mini-αB 73-86	DRFSVNLDVKHFSP	6.7	ADH	0	51
Mini-αB 73-84	DRFSVNLDVKHF	6.7	ADH	0	51
Mini-αB 73-82	DRFSVNLDVK	5.9	ADH	0	51
αB-Chimeric peptide–CP3	DRFSVNLDVKHFEEKPTSAPSS	5.5	ADH	30	PR
αB-Chimeric peptide–CP4	DRFSVNLDVKHFEEKPAVTAAPKK	8.4	ADH	40	PR
RG Mini-αB GR	RGFSVNLDVKHFSPEELKGR	8.6	ADH	20	PR
RG Mini-αB 89-92 GR	RGFSVNLDVKHFSPEEGR	6.7	ADH	0*	PR
RG Mini-αB 91-92 GR	RGFSVNLDVKHFSPEELKGR	8.6	ADH	50	PR
Mini-αB 75-92	FSVNLDVKHFSPEELKVK	6.7	ADH	70	51
Mini-αB 77-92	VNLDVKHFSPEELKVK	6.7	ADH	0	51
Mini-αB 75-92 (D82A)	FSVNLAVKHFSPEELKVK	8.5	ADH	24	51
Mini-αB 75-92 (H85A)	FSVNLDVKAFSPEELKVK	6.1	ADH	69	51
Mini-αB 73-92 (H85A)	DRFSVNLDVKAFSPEELKVK	6.7	ADH	0	51
Mini-αB (all D forms)	DRFSVNLDVKHFSPEELKVK	6.7	ADH	50	PR
RG Mini-αB GR (80 D-Asp)	RGFSVNL{D-Asp}VKHFSPEEGR	6.7	ADH	10	PR
Mini-αB 89-92 (80 D-Asp)GR	RGFSVNL{D-Asp}VKHFSPEELKGR	8.6	ADH	80	PR
Mini-αB 75-92 (80 D-Asp)	FSVNL{D-Asp}VKHFSPEELKVK	6.7	ADH	50	PR

The residues shown in color are derived from αB-crystallin chaperone region (αB73-92).

*
indicates peptide by itself aggregated.

[1]The chaperone activity of peptides measured using methods described earlier [31, 61] was compared against Mini-αB chaperone activity. The chaperone activity of mini-αB was considered 100.

PR–Present study