αB-Crystallin Selectively Targets Intermediate Filament Proteins during Thermal Stress

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PURPOSE. αB-Crystallin is a small heat shock protein (sHsp) expressed at high levels in the lens of the eye, where its molecular chaperone functions may protect against cataract formation in vivo. The purpose of this study was to identify protein targets for the sHsp αB-crystallin in lens cell homogenates during conditions of mild thermal stress.

METHODS. The authors report the use of a fusion protein, maltose-binding protein αB-crystallin (MBP-αB), immobilized on amylose resin as a novel method for isolating endogenous αB-crystallin-binding proteins from lens cell homogenates after mild thermal stress.

RESULTS. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western immunoblot analyses showed selective interactions in lens cell homogenates between MBP-αB and endogenous αA- and αB-crystallins, the lens-specific intermediate filament proteins phakinin (CP49) and filensin (CP115), and vimentin during a mild 20-minute heat shock at 45°C. No interactions were observed with the β- or γ-crystallins, or the cytoskeletal proteins actin, α-tubulin, and spectrin, although these proteins were present in lens cell homogenates. In contrast, γ-crystallin and actin interacted with MBP-αB at 45°C only in their purified states. The results obtained with MBP-αB were confirmed by immunoprecipitation reactions in which immunoprecipitation of phakinin and filensin.

CONCLUSIONS. In the lens the sHsp αB-crystallin may selectively target intermediate filaments for protection against unfolding during conditions of stress. (Invest Ophthalmol Vis Sci. 1999;40:951-958)
an interaction would have functional significance. In this study endogenous aB-crystallin-binding proteins were isolated from lens cell homogenates using their affinity for the fusion protein MBP-aB during mild thermal stress. MBP-aB assembles into a high-molecular-weight complex similar to native aB-crystallin and other sHsps. The chaperone activity of MBP-aB in aggregation assays using alcohol dehydrogenase at 37°C and citrate synthase at 45°C (Muchowski PJ, Clark JI, unpublished data) as target proteins was indistinguishable from native aB-crystallin. The chaperone activity of MBP-aB in the refolding of denatured citrate synthase was identical with that of native aB-crystallin (unpublished data). In the present study, the selectivity of MBP-aB for endogenous aA- and aB-crystallins, and the intermediate filament proteins phakinin, filensin, and vimentin in lens cell homogenates was shown under mild thermal stress at 45°C. Immunoprecipitation of native aB-crystallin from heat-shocked lens cell homogenates result in the coprecipitation of phakinin and filensin. The results suggest that in lens cells the target proteins for the sHsp aB-crystallin may be intermediate filaments and that the aB-crystallin–cytoskeletal interaction is important for maintaining lens cell structure and transparency.

MATERIALS AND METHODS

Purification of MBP-aB and Preparation of Bovine Lens Cell Homogenates

Human aB-crystallin was expressed, purified, and characterized functionally as a soluble fusion protein, MBP-aB, as described previously. Fresh bovine lenses were obtained from a local abattoir, and divided into cortex and nucleus, as described previously. The interface between the cortex and the nucleus was determined by the appearance of a cold cataract. To separate the proteins into water-soluble and insoluble fractions, the lens cell homogenates were homogenized in a handheld tissue homogenizer, diluted into buffer A (20 mM Tris-HCl [pH 7.4], 20 mM NaCl, and 1 mM EDTA), mixed thoroughly, and centrifuged at 10,000g for 30 minutes at 4°C. The supernatants, representing the water-soluble fraction, were removed and placed into separate tubes. The water-insoluble pellets were resuspended in 4 ml buffer A containing 8 M urea. After resuspension the water-insoluble fraction was centrifuged a second time at 10,000g for 30 minutes at 4°C. The supernatant was then diluted 10-fold in buffer A. Concentrations of MBP-aB and bovine soluble and insoluble lens protein fractions were determined by Bradford protein assays using bovine serum albumin as a protein standard (Bio-Rad).

Interaction of MBP-aB with Soluble and Insoluble Lens Proteins during Heat Shock

Fifty micrograms of each lens cell homogenate (soluble and insoluble nucleus and cortex) was placed in 200 µl buffer B (20 mM Tris-HCl [pH 7.4], 200 mM NaCl, and 1 mM EDTA) in the presence and absence of 50 µg MBP-aB, and heat shocked for 20 minutes at 45°C. The final concentration of urea remaining was approximately 0.6 M. Lens cell homogenates that were heat shocked in the presence of MBP-aB were similarly adsorbed to 100 µl amylose resin pre-equilibrated in buffer B. The reaction mixtures were incubated for 10 minutes at 23°C to allow for complete adsorption of MBP-aB to the amylose resin. The reaction mixtures were then centrifuged at 12,000g for 5 minutes at 23°C. After discarding the supernatant, the amylose resin containing MBP-aB and associated proteins was washed with 200 µl buffer B. This process was repeated two times. The third wash was performed with 100 µl elution buffer (buffer B containing 10 mM maltose) to release MBP-aB and its associated proteins from the amylose resin.

Identification of Soluble and Insoluble Lens Proteins Interacting with MBP-aB during Heat Shock by SDS-PAGE and Western Blot Analysis

Proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 4% to 12% Bis-Tris polyacrylamide electrophoretic gels run in the presence of 0.1% sodium dodecyl sulfate and MES or MOPS buffer (NOVEX, Encinitas, CA) and were stained with Coomassie blue (R-350; Pharmacia, Piscataway, NJ). Gels were loaded with equal volumes of the elution buffer from the reaction mixtures described earlier. Proteins were electrophoretically transferred to either nitrocellulose or polyvinylidene difluoride membranes using a kit (Blot Module II; Novex). The following were used as primary antibodies: rabbit anti-bovine phakinin (CP49) and filensin (CP115) antisera; rabbit anti-bovine aA-, aB-, and a-crystallin antisera; monoclonal anti-actin; a-tubulin and vimentin, and polyclonal anti-spectrin (Sigma). For immunodetection, alkaline phosphatase conjugated to goat anti-rabbit or anti-mouse IgG antibody and 5-bromo-4-chloro-3'-indolylphosphate and p-nitro blue tetrazolium chloride (Bio-Rad) were used.

Identification of Insoluble Lens Proteins Interacting with Native Bovine aB-Crystallin during Heat Shock by Immunoprecipitation

One hundred micrograms of each insoluble lens cell homogenate was incubated for 20 minutes at 45°C in 500 µl buffer B. The following antibodies were then used for immunoprecipitation reactions: rabbit anti-recombinant human aB-crystallin antisera (1:20), mouse anti-chicken vinculin monoclonal antibody (1:100 of stock solution; Sigma), and rabbit anti-human gastric mucin monoclonal antibody (1:200 of stock solution; Sigma). The various antibodies were incubated with heat-shocked insoluble lens cell homogenates for 10 minutes at 23°C. Subsequently, 50 µl protein A Sepharose (Pharmacia) pre-equilibrated in buffer B was added to the samples and incubated for 10 minutes at 23°C. Samples were centrifuged in a microfuge at 12,000g for 5 minutes at 23°C. Samples were next washed twice with 200 µl buffer B. Proteins bound to the protein A Sepharose were released with 100 µl 0.1% SDS, resolved by SDS-PAGE, as described above, transferred to polyvinylidene difluoride membranes (Immobilon; Amersham; Arlington Heights, IL), and probed with anti-phakinin and anti-filensin antisera (as described). For immunodetection, horse radish peroxidase conjugated to goat anti-rabbit or anti-mouse IgG antibody was used. Bound protein–IgG complexes were detected by chemiluminescence with enhanced chemiluminescence reagent (Amersham), followed by autoradiography.
To characterize the temperature dependence of the interaction between MBP-αB and lens proteins, reactions were performed as described, with the exception that incubations at temperatures of 4°C, 23°C, 37°C, and 45°C were performed. Reaction mixtures were analyzed by SDS-PAGE and western immunoblot analysis, as described. Densitometric analysis of scanned gels and blots was performed as follows. Dried gels or blots were digitized and stored as 8-bit/pixel gray-scale image files. A computer program (NIH IMAGE 1.58; National Institutes of Health, Bethesda, MD) was used for quantification of relative band densities at different incubation temperatures. In Table 1, the amount of total lens protein associated with MBP-αB at 37°C and 45°C for each lens fraction is shown as an approximate proportion of the amount associated at 23°C. More specifically, each gel lane was scanned and analyzed for total band density (in pixels). The amount of protein binding to MBP-αB was calculated and is reported as an approximate proportion by dividing the total band density detected at 37°C and 45°C by the total band density detected at 23°C. Similarly, the total amount of bovine lens αB-crystallin associated with MBP-αB at 37°C and 45°C for each lens fraction is shown as a proportion of the amount associated at 23°C in Table 1.

**RESULTS**

**Interaction of MBP-αB with Soluble and Insoluble Lens Proteins during Heat Shock**

Purified MBP-αB was used to characterize potential interactions with proteins in lens cell homogenates during thermal stress. Fresh calf lenses were homogenized and separated into four distinct protein fractions: insoluble and soluble nucleus (representing the center of the lens), and insoluble and soluble cortex (representing the periphery of the lens). To characterize the interactions between MBP-αB and proteins from the lens cell homogenates during thermal stress, the following experimental protocol was followed (Fig. 1). The four fractions of lens cell homogenates were incubated at 45°C for 20 minutes in the presence and absence of an equal amount (wt/wt) of MBP-αB. MBP-αB, heat shocked in the presence of the lens cell homogenates, was then adsorbed to amylose affinity resin in buffer B. Fractions that were heat shocked at 45°C for 20 minutes in the absence of MBP-αB were subsequently mixed with an equal amount of MBP-αB preadsorbed to amylose affinity resin equilibrated in buffer B. Fractions that were heat shocked at 45°C for 20 minutes in the absence of MBP-αB were subsequently mixed with an equal amount of MBP-αB preadsorbed to amylose affinity resin at room temperature. After extensive washing, the immobilized MBP-αB/lens protein complexes were released from the amylose resin by a wash with buffer containing maltose. Proteins interacting with MBP-αB were then analyzed by SDS-PAGE and western immunoblot analysis. Extensive washing ensured that nonspecific interactions between the lens cell homogenates and the amylose resin, alone or with MBP immobilized on the amylose resin, were never observed (data not shown).

**Temperature-Dependent Interactions between MBP-αB and Lens Proteins**

To characterize the temperature dependence of the interaction between MBP-αB and lens proteins, reactions were performed as described, with the exception that incubations at temperatures of 4°C, 23°C, 37°C, and 45°C were performed. Reaction mixtures were analyzed by SDS-PAGE and western immunoblot analysis, as described. Densitometric analysis of scanned gels and blots was performed as follows. Dried gels or blots were digitized and stored as 8-bit/pixel gray-scale image files. A computer program (NIH IMAGE 1.58; National Institutes of Health, Bethesda, MD) was used for quantification of relative band densities at different incubation temperatures. In Table 1, the amount of total lens protein associated with MBP-αB at 37°C and 45°C for each lens fraction is shown as an approximate proportion of the amount associated at 23°C. More specifically, each gel lane was scanned and analyzed for total band density (in pixels). The amount of protein binding to MBP-αB was calculated and is reported as an approximate proportion by dividing the total band density detected at 37°C and 45°C by the total band density detected at 23°C. Similarly, the total amount of bovine lens αB-crystallin associated with MBP-αB at 37°C and 45°C for each lens fraction is shown as a proportion of the amount associated at 23°C in Table 1.

**Interaction of MBP-αB and Purified Proteins during Heat Shock**

Fifty micrograms of purified recombinant human αB-crystallin, bovine γB- and γD-crystallins, and the cytoskeletal proteins actin and vimentin (Sigma) were substituted for lens cell homogenates and treated in the experimental protocol, as described earlier. Proteins interacting with MBP-αB were analyzed by SDS-PAGE, as described above.
Identification of Soluble and Insoluble Lens Proteins Interacting with MBP-\(\alpha\B)-Crystallin during Heat Shock by SDS-PAGE Analysis

Western immunoblot analyses were performed to identify the proteins in lens cell homogenates interacting with MBP-\(\alpha\B) during heat shock at 45°C (Fig. 3). Antisera specific to bovine lens \(\alpha\A-\) and \(\alpha\B-crystallins reacted positively with proteins interacting with MBP-\(\alpha\B) in all four lens fractions at the molecular masses expected for bovine lens \(\alpha\A-\) and \(\alpha\B-crystallins (~20\,kDa); Fig. 3, lanes 4, 7, 10, 13). In contrast, antisera raised against bovine \(\beta\)- and \(\gamma\)-crystallins did not react with proteins associated with MBP-\(\alpha\B) in any of the lens fractions, although these proteins were detected in lens cell homogenates (data not shown). MBP-\(\alpha\B) had to be present with the lens cell homogenates during heat shock to form stable complexes with the lens proteins (Fig. 3, compare lanes 3 and 4, 6 and 7, 9 and 10, 12 and 13).

Identification of Insoluble Lens Proteins Interacting with Native Bovine \(\alpha\B-Crystallin during Heat Shock by Immunoprecipitation

To confirm that the proteins interacting with MBP-\(\alpha\B) were similar to those bound to native bovine \(\alpha\B-crystallin in heat shocked lens cell homogenates, immunoprecipitation experiments were conducted. Insoluble lens cell homogenates were heat shocked at 45°C, as described in the experimental protocol (Fig. 1), followed by immunoprecipitation of native bovine \(\alpha\B-crystallin (Fig. 4). Proteins coprecipitating with native bovine \(\alpha\B-crystallin were resolved by SDS-PAGE and identified by Western immunoblot analysis. Immunoprecipitation of native bovine \(\alpha\B-crystallin from heat shocked lens cell homogenates resulted in the coprecipitation of phakinin (Fig. 4A, lanes 2, 6). Immunoprecipitation in the absence and presence of two control antibodies did not result in the coprecipitation of phakinin (Fig. 4A, lanes 1, 3, 4, 5, 7, 8). Immunoprecipitation of native bovine \(\alpha\B-crystallin from heat shocked lens cell homogenates resulted in the coprecipitation of filensin (Fig. 4B, lanes 2, 6). Immunoprecipitation in the absence and presence of two control antibodies resulted in the coprecipitation of low amounts of proteins associated with MBP-\(\alpha\B) in any of the lens fractions, although these proteins were detected in lens cell homogenates (data not shown). MBP-\(\alpha\B) had to be present with the lens cell homogenates during heat shock to form stable complexes with the lens proteins.
TABLE 1. Temperature-Dependent Interactions between MBP-αB and Lens Proteins: Densitometric Analysis

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Western immunoblots (bovine αB-crystallin only)†

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* SDS-PAGE gels shown in Figure 5A were analyzed by densitometry, as described in the Materials and Methods section. The total amount of lens proteins associated with MBP-αB at 37°C and 45°C for each lens fraction is shown as a proportion of the amount associated at 23°C. Western immunoblots shown in Figure 5B were analyzed by densitometry, as described in the Materials and Methods section. The total amount of bovine αB-crystallin associated with MBP-αB at 37°C and 45°C for each lens fraction is shown as a proportion of the amount associated at 23°C.

Temperature-Dependent Interactions between MBP-αB and Lens Proteins

The four fractions of lens cell homogenates were incubated at 4°C, 23°C, 37°C, or 45°C in the presence and absence of MBP-αB to assess the influence of temperature on the interactions between MBP-αB and lens proteins. The proteins interacting with MBP-αB at different temperatures were isolated as described in the experimental protocol (Fig. 1). Densitometric analysis of scanned polyacrylamide gels showed that in the insoluble lens nucleus fraction, approximately three times more total lens protein associated with MBP-αB at 37°C than at 23°C, and approximately 11 times more total lens protein associated with MBP-αB at 45°C than at 23°C (Table 1). In all lens fractions, the total amount of lens protein interacting with MBP-αB was proportional to the incubation temperature with no detectable binding observed at 4°C and the highest amount of binding observed at 45°C (Fig. 5A; Table 1). Densitometry of scanned immunoblots using antisera specific for bovine αB-crystallin was performed to determine the relative amount of native bovine lens αB-crystallin interacting with MBP-αB at different incubation temperatures (Fig. 5B; Table 1). In the soluble lens cortex fraction, approximately four times more αB-crystallin associated with MBP-αB at 37°C than at 23°C, and approximately 16 times more αB-crystallin associated with MBP-αB at 45°C than at 23°C (Table 1). In all lens fractions, the total amount of native bovine αB-crystallin interacting with MBP-αB was proportional to the incubation temperature (Table 1).

Interaction of MBP-αB with Purified Proteins during Heat Shock

The interactions between MBP-αB and proteins in lens cell homogenates were next compared with the interactions between MBP-αB and purified proteins during heat shock. Purified human αB-crystallin, bovine γB- and γD-crystallins, and the cytoskeletal proteins actin, spectrin, α-tubulin, and vimentin were tested for interactions with MBP-αB during heat shock at 45°C. The purified proteins interacting with MBP-αB at 45°C were isolated as described in the experimental protocol (Fig. 1). SDS-PAGE analysis of proteins interacting with MBP-αB showed that all the purified proteins formed stable complexes with MBP-αB at 45°C (Fig. 6). The interaction between purified vimentin and MBP-αB during heat shock decreased the yield of MBP-αB eluted from the amylose resin (Fig. 6, lane 6), indicating that

![Figure 4](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933211/ on 10/15/2018)
purified vimentin decreased the affinity between MBP-αB and the amylose resin. Similar to the results observed with lens cell homogenates, the presence of MBP-αB before heat shock was required to form stable complexes with the purified proteins (data not shown). In contrast to the results observed with lens cell homogenates, purified bovine γB- and γD-crystallins and actin interacted with MBP-αB at 45°C. Several other purified proteins including alcohol dehydrogenase, bovine serum albumin, citrate synthase, and lysozyme were tested in the experimental system, and all purified proteins interacted with MBP-αB during heat shock at 45°C (data not shown). Nonspecific binding of purified proteins to the amylose resin or to MBP coupled to amylose resin was not observed (data not shown).

DISCUSSION

In this study an MBP-αB fusion protein was used in a novel assay for identifying αB-crystallin-binding proteins in lens cell homogenates under conditions of mild thermal stress. The results suggest that αB-crystallin selectively targets intermediate filament proteins in vivo, in contrast to its apparent nonselective interactions with purified proteins in vitro. The similarities between the activity of MBP-αB and native human αB-crystallin as molecular chaperones indicates that the results have functional significance.26

![Figure 5](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933211/)

**Figure 5.** Temperature-dependent interactions between MBP-αB and lens proteins. (A) SDS-PAGE analysis of lens proteins interacting with MBP-αB after a 20-minute incubation at 4°C, 23°C, 37°C, and 45°C. Shown are scans of Coomassie blue-stained polyacrylamide electrophoretic gels run in the presence of 0.1% SDS. Molecular weight standards are listed in lane 1. Lens fractions are listed as IN, insoluble nucleus; IC, insoluble cortex; SN, soluble nucleus; and SC, soluble cortex. Lanes 2, 7, 12, 17 contained proteins found in lens cell homogenates (starting control proteins labeled EX are extract). Lanes 3, 8, 13, 18 contained lens cell homogenate proteins interacting with MBP-αB after a 20-minute incubation at 4°C. Lanes 4, 9, 14, 19 contained lens cell homogenate proteins interacting with MBP-αB after a 20-minute incubation at 23°C. Lanes 5, 10, 15, 20 contained lens cell homogenate proteins interacting with MBP-αB after a 20-minute incubation at 37°C. Lanes 6, 11, 16, 21 contained lens cell homogenate proteins interacting with MBP-αB after a 20-minute incubation at 45°C. (B) Western immunoblot analysis of bovine lens αB-crystallin associated with MBP-αB after a 20-minute incubation at 4°C, 23°C, 37°C, and 45°C. Proteins identical with those shown in (A) were transferred to polyvinylidene difluoride membranes and reacted with ant-αB-crystallin antisera (~20 kDa). The lanes are numbered identically with those shown in (A). Shown is an alkaline-phosphatase development of bound αB-crystallin-IgG complexes. The interactions between MBP-αB and lens proteins were strongly temperature dependent.

![Figure 6](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933211/)

**Figure 6.** SDS-PAGE analysis of purified proteins interacting with MBP-αB during heat shock at 45°C. Shown is a scan of a Coomassie blue-stained polyacrylamide electrophoretic gel run in the presence of 0.1% SDS. Molecular weight standards are listed in lane 1. Lanes 2 through 6 contained purified proteins interacting with MBP-αB after a 20-minute incubation at 45°C (see experimental protocol). Lanes 2 through 6 contained MBP-αB interacted with purified recombinant human αB-crystallin (~20 kDa), bovine γB-crystallin (~20 kDa), bovine γD-crystallin (~20 kDa), actin (~42 kDa), and vimentin (~54 kDa), respectively. In contrast to the results with cell homogenates, MBP-αB interacted with all purified proteins tested at 45°C, including γ-crystallins and the cytoskeletal protein actin.
Although approximately 90% of all the proteins found in the mammalian lens are comprised of the α- and β-γ-crystallin families, MBP-αB interacted only with αA- and αB-crystallins, and not with β- or γ-crystallins in lens cell homogenates at 45°C. α-Crystallins isolated from lenses are high-molecular-weight complexes consisting of the αA subunits and αB subunits, and it has been shown that the subunits are interchangeable within the high-molecular-weight complexes. Strikingly, MBP-αB assembled into a high-molecular-weight complex similar to αA- and αB-crystallins, despite the presence of an amino terminal approximately M₄, 43-kDa MBP fusion tag. At the temperature used in the experimental system, 45°C, it is possible that αA- and αB-crystallins exchanged subunits with MBP-αB and were not trapped as unfolding intermediates by MBP-αB. Future investigations will be required to analyze whether MBP-αB undergoes subunit exchange similar to native αA- and αB-crystallins. In contrast to the results using cell homogenates, interactions were observed between MBP-αB and purified bovine γ-crystallins at 45°C. In lens cells γ-crystallins may be interacting with proteins or cofactors other than αB-crystallin. In the lens γ-crystallins are normally in close contact with highly concentrated cytoplasmic proteins, and the biochemical properties of γ-crystallins may be altered during or after isolation from lens cells.

MBP-αB had to be present with the lens cell homogenates during heat shock to form stable complexes with the lens proteins. This result is consistent with those in previous studies using α-crystallins and sHsps, which also showed that α-crystallins and sHsps must be present during heat shock to form stable complexes with unfolding target proteins. In the absence of MBP-αB, unfolding lens proteins may have aggregated so that sites for interaction with MBP-αB were no longer available. Similar to other sHsps, MBP-αB may interact only with early unfolding intermediates.

MBP-αB selectively targeted the lens-specific intermediate filament proteins phakinin, filensin, and vimentin at 45°C in lens cell homogenates, judged by SDS-PAGE and western immunoblot analysis. Although the selective targeting of intermediate filaments by MBP-αB may simply reflect the temperature dependence of target protein unfolding, it was striking that only phakinin, filensin, and vimentin associated with MBP-αB at 45°C in lens cell homogenates.

Cytoskeletal proteins comprise only 2% to 4% of the total lens protein. Despite their relatively low expression levels compared with the crystallins, the importance of cytoskeletal proteins in the development and maintenance of transparent lens cell structure has been reported. The interactions between MBP-αB and cytoskeletal proteins may have functional significance. The assembly of phakinin and filensin into a beaded filament structure in vitro occurred only in the presence of α-crystallins, and in the in vitro assembly of vimentin and GFAP was inhibited by α-crystallins. Exposure of cells in culture to heat shock disrupted the organization of intermediate filaments, and heat shock proteins such as α-crystallins may mediate the successful recovery of the cells after heat shock. In lenses, interactions between αB-crystallin and cytoskeletal proteins may be critical for maintaining the structural integrity of lens cells during heat shock or other forms of stress. Furthermore, the interactions between MBP-αB and cytoskeletal proteins have broad implications for the nonlens functions of αB-crystallin in heart and skeletal muscle, brain, kidney, placenta, and lung. αB-Crystallin is upregulated dramatically in neurodegenerative diseases that include multiple sclerosis, Alzheimer's disease, Alexander's disease, and Creutzfeldt-Jakob disease.

The colocalization of αB-crystallin with actin and intermediate filaments in several human pathologic conditions suggests a general function for αB-crystallin in the cellular response to stresses associated with these human diseases. In nonlens tissues, αB-crystallin–cytoskeletal protein interactions may help maintain the structural integrity of cells during heat shock. Disruption of αB-crystallin–cytoskeletal protein interactions may be implicated in pathologic conditions including those that involve cataract formation.

In this study an interaction between MBP-αB and actin in lens cell homogenates was not detected. This finding was unexpected, because α-crystallins have been shown to bind actin in vitro, stabilizing the actin filaments and preventing cytchalasin-induced depolymerization. α-Crystallins have also been shown to interact with actin and desmin filaments in vivo in cardiac tissue. Functionally, genetic experiments in yeast have shown that expression of HSP42, an sHsp that contains an α-crystallin motif, promotes maintenance of the actin cytoskeleton. The results in this study suggest that the interaction of αB-crystallin with proteins such as actin may be modulated differently in vivo in lens cells and in vitro, a point that should be considered in future investigations. Current investigations are under way to elucidate whether αB-crystallin selectively targets intermediate filament proteins in nonlens cells for protection against unfolding during thermal stress.

In conclusion, we have shown that MBP-αB interacted selectively with intermediate filament proteins in lens cell homogenates at 45°C. Accumulating evidence, including the experiments in this study, suggest that the molecular chaperone activities of the sHsp αB-crystallin in vivo may involve cytoskeletal protein interactions in the maintenance of protein function, cell structure and lens transparency.

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